Volume 29, Number 6, June, 1957

The Yale Journal of Biology & Medicine

565 **NEURAL CONTROL OF HUNGER, APPETITE, AND SATIETY** *John R. Brobeck*

575 **ACTIVITY IN STARVATION** *J. A. F. Stevenson and R. H. Rixon*

583 **SUICIDE AMONG COLLEGE STUDENTS** *Henry M. Parrish*

596 **BIOCHEMICAL ASPECTS OF PANCREATITIS** *Harris Busch*

613 **GROWTH REQUIREMENTS OF *N. GONORRHOEAE*** *Philip J. Griffin and Sidney V. Rieder*

622 **EVENTS**

628 **BOOK REVIEWS**

637 **INDEX TO VOLUME 29**

New Potentiated Pain Relief

A.P.C. WITH Demerol®

Tablets

Each tablet contains

Aspirin	200 mg. (3 grains)
Phenacetin	150 mg. (2½ grains)
Caffeine	30 mg. (½ grain)
Demerol® hydrochloride	30 mg. (½ grain)

Average Adult Dose... 1 or 2 tablets repeated in three or four hours as needed.

- ***marked potentiation of analgesia***

plus

- *mild sedation*
- *antispasmodic action*
- *antipyretic action*
- *no constipation*
- *no interference with micturition*

"Such a combination has proved clinically to be far more effective and no more toxic than equivalent doses of any of these used singly."

Bonica, J.J.; and Backup, P.H. (Tacoma General Hospital, Washington): *Northwest Med.*, 54:22, Jan., 1955.

Supplied in bottles of 100 tablets.

NARCOTIC BLANK REQUIRED

Winthrop LABORATORIES | NEW YORK 18, N. Y. • WINDSOR, ONT.

Demerol (brand of meperidine), trademark reg. U. S. Pat. Off.

CONTENTS

565 **NEURAL CONTROL OF HUNGER, APPETITE, AND SATIETY** *John R. Brobeck*

575 **ENVIRONMENTAL TEMPERATURE AND DEPRIVATION OF FOOD AND WATER ON THE SPONTANEOUS ACTIVITY OF RATS** *J. A. F. Stevenson and R. H. Rixon*

585 **EPIDEMIOLOGY OF SUICIDE AMONG COLLEGE STUDENTS** *Henry M. Parrish*

596 **BIOCHEMICAL ASPECTS OF PANCREATITIS** *Harris Busch*

613 **A STUDY ON THE GROWTH REQUIREMENTS OF *NEISSERIA GONORRHOEAE* AND ITS CLINICAL APPLICATION** *Philip J. Griffin and Sidney V. Rieder*

622 **EVENTS**

628 **BOOK REVIEWS**

637 **INDEX TO VOLUME 29**

THE YALE JOURNAL
OF BIOLOGY AND MEDICINE

Founded in 1928.

George H. Smith; *Editor-in-Chief, 1928-49; Editor-in-Chief Emeritus, 1949-52.*

BOARD OF EDITORS

Faculty

Paul B. Beeson; Frederick G. Kilgour, *Managing Editor;* James W. Lewis, *Advertising Manager;* Averill A. Liebow; Vernon W. Lippard, *Chairman;* C. N. H. Long; Roy Schafer; Max Taffel; Elizabeth H. Thomson, *Assistant Editor;* Talbot H. Waterman

Students

Roger P. Atwood; Richard I. Breuer; Ronald H. Fishbein; Jack Peter Green; Stanley I. Harris; Peter H. Klopfer; Myron M. Lotz; N. Ronald Morris; Robert S. Neuwirth; James A. O'Neill, Jr.; Lisa A. Steiner; George E. Watson, 3rd

---

Subscriptions. THE YALE JOURNAL OF BIOLOGY AND MEDICINE is published in September, November, December, February, April, and June from 333 Cedar Street, New Haven 11, Connecticut, U.S.A. The subscription price for one volume of six issues is $7.50 in the United States and other countries. For students registered in any school of Yale University, the subscription price is $4.00 per volume. Single copies are $1.50 each except for special issues.

Entered as second-class matter January 11, 1929, at the post office at New Haven, Connecticut, under the Act of March 3, 1879.

PRINTED BY THE VAN DYCK PRINTING CO., NEW HAVEN, CONN.

Scope. THE YALE JOURNAL OF BIOLOGY AND MEDICINE publishes original contributions in all fields of medicine, the fields of biology that are related to medicine, and in the history and teaching of these subjects.

Mailing address. Manuscripts should be addressed to THE YALE JOURNAL OF BIOLOGY AND MEDICINE, 333 Cedar Street, New Haven 11, Connecticut, U.S.A. All manuscripts should be mailed flat.

Preparation of copy. Papers should be typed in double or triple space, with references typed in double space on a separate sheet. For the convenience of authors, as well as of the editors, certain conventions in style of citation have been adopted, as follows: References will be listed alphabetically by author and numbered consecutively. In the text they will be cited by superior number without parentheses. The order of citation of journal articles will be: author's name (surname followed by initials or forename if there is no middle initial), title of article, abbreviated title of journal, year, volume number, and inclusive pages of article. Titles of journals will be abbreviated in accordance with *World Medical Periodicals* published by WHO and UNESCO, 1953, and based on the *World List of Scientific Periodicals,* 1952. (In case these publications are not available to the author, full titles of journals should be submitted.) References to books will include the author's name, the title of the book, and the place of publication, publisher, and date, followed by the page or pages containing the information to which reference is made. Authors of historical papers may use the conventional footnote style.

Lucké, Baldwin and Rea, M. H.: Studies on aneurysm. *J. Amer. med. Ass.*, 1921, *77*, 935-940.

Barcroft, Joseph: *The respiratory function of the blood.* Cambridge, Cambridge University Press, 1914, pp. 58-61.

Illustrations. All illustrations and tabular matter must be in finished form for the printer. Illustrations must not be larger than 8½ by 11 inches; if the original is larger, it should be photographically reduced on a glossy print. Line cuts will usually be reduced for printing to four inches or less in width. The artist should be informed of the anticipated reduction before the drawing is made. All illustrative material should have titles, and the legends should be on the material itself as well as typed on a separate sheet.

Costs chargeable to authors. The number of free illustrations will vary with the length of the paper, but usually only two pages of halftones will be allowed free. Costs for additional halftones will be charged to the author. Special typography, including tables and formulas, in excess of 10 per cent of composition costs are chargeable to the author. Corrections in galley proof in excess of 5 per cent of original composition costs will also be charged to the author. Page proof will not normally be sent, but in the event that an author makes any changes from copy after galley proof has been submitted, the entire cost will be charged to him.

Managing Editor and Production Manager checking copies of Scope Weekly as they come off press.

Covering the medical beat

SCOPE Weekly, Upjohn's medical newspaper for physicians in the United States and Alaska, stands midway in its second year of publication as the most popular periodical of its type.

Designed and operated as a service to members of the medical profession, SCOPE Weekly provides its readers with global medical news and features coverage on a par with high-ranking metropolitan dailies. Hundreds of correspondents throughout the world beam contributions to the paper's central office in New York City, where a staff of seasoned physicians, medical writers, and journalists assemble, edit, and produce 52 issues a year.

Although SCOPE Weekly is the property of The Upjohn Company, decisions involving editorial policy and content are handled by its staff, Physicians News Service, and not by its owner. This complete objectivity in reporting happenings on the medical scene has helped to build for SCOPE Weekly a reputation as an authoritative source of news and information of interest to the profession.

Upjohn

KALAMAZOO, MICHIGAN

Medicine...
Designed for health...
Produced with care

JOHN R. BROBECK*

Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia

NEURAL CONTROL OF HUNGER, APPETITE, AND SATIETY**

Hunger, appetite, and satiety are often regarded as problems in the physiology of digestion. In recent years, however, they have been studied more and more commonly as functions of the brain. This latter point of view, i.e., neurophysiology, is used in the paper which follows, and the regulation of food intake is here described in terms of mechanisms and reactions that characterize the nervous system.

In higher animals the brainstem, including the nuclei and primary connections of the cranial nerves, is the level of the nervous system most intimately concerned with food intake. The basic patterns are probably reflex in nature. They require the integrated motor activity of the trigeminal, facial, glossopharyngeal, vagal, and hypoglossal nerves, while the sensory components of these, as well as many other nerves, also play their part. For almost any animal, including one which has just eaten, food is a stimulus for reflex responses; when there occurs an adequate sensory stimulation, the animal is inclined to move toward the food, to investigate, eat, and swallow it (Table 1). Reflexes of attention, approach, examination, incorporation, and of rejection appear to be important. Little is known of these mechanisms, however, and it is not even clear just how much of feeding behavior can be designated as reflex, and how much may be either conditioned responses or some other type of more highly organized activity. Yet in spite of this ignorance, I have chosen to begin with this attempt to identify and classify the reflexes, because Sherrington and others successfully used this method in their studies of posture and locomotion.

The first part of this paper is a brief description of the behavior of animals with lesions at selected levels of the neuraxis, and of unanesthetized animals during stimulation of discrete areas within the brain. Most of the data are taken from studies on mammals, since there seems to be little information relating the nervous system and feeding in lower forms. Experiments by Miller and Sherrington[16] and by Bazett and Penfield,[4] using

* Professor of Physiology and Chairman of the Department.

** A paper read at a symposium of the International Physiological Congress, August 1, 1956, in Brussels. Attendance at the Congress was made possible by a grant from the National Science Foundation.

Received for publication March 4, 1957.

Copyright 1957 by THE YALE JOURNAL OF BIOLOGY AND MEDICINE, INC.

decerebrated cats, showed that simple feeding responses are possible after removal of much of the mesencephalon and all of the more rostral portions of the brain. Their decerebrated animals were capable of reflex chewing and swallowing and reflex rejection of certain materials. Bazett and Penfield noted purring after their cats were fed, which suggests that a certain degree of "satiety" can occur even at the reflex or segmental level.

The function of the hypothalamus, the next higher level, is assumed to be a quantitative one, as if this part of the brain adjusts energy intake to energy expenditure. The medial hypothalamus is believed to take part in

TABLE 1. CLASSIFICATION OF FEEDING REFLEXES, ON SENSORY BASIS (LEFT-HAND COLUMN) AND BEHAVIORAL BASIS (RIGHT-HAND COLUMN). (From J. R. Brobeck, Gastroenterology, 1957, *32*, 169-174.

Sensory basis	*Behavioral basis*
Visual reflexes	Reflexes of attention
Olfactory reflexes	Reflexes of approach
Auditory reflexes	Reflexes of examination
Tactile reflexes	Reflexes of incorporation
Gustatory reflexes	Reflexes of rejection
Enteroceptive reflexes	

reactions of satiety, while the lateral portions are responsible for appetite.[3] After destruction of the lateral regions, animals fail to eat; in some cases the failure is absolute, in others it is only relative.[9, 25] Medial lesions, by contrast, lead to overeating and obesity in all species studied, including man. When the lateral mechanism is stimulated in unanesthetized animals, feeding occurs (Brügger,[7] Larson,[21] and Anand and his associates[2]). Stimulation of the medial region, as well as of certain other portions of the brain, induces what appears to be satiety; Olds has observed this,[18] and others have confirmed his results. The interaction of these two hypothalamic regions may account for most of the quantitative aspects of regulation of food intake, since the cerebral cortex may very well affect the appetite by way of these hypothalamic mechanisms. It is conceivable that the lateral hypothalamus or appetite mechanism serves to facilitate feeding reflexes, while the medial hypothalamus or satiety mechanism acts to inhibit the reflexes. In a fasting condition the lateral portion would be active, the medial one quiet, with a resulting high appetite and low satiety. But after feeding, the lateral portion would be quiet and the medial one active—low appetite and high satiety. When the medial mechanism is injured and hyperphagia follows, any remaining regulation probably occurs through variations in activity of the

lateral portion, that is, through changes only in appetite. This may explain why appetite is so labile or changeable in animals with hyperphagia, as Kennedy,[19] Stevenson,[17] and Teitelbaum[24] have observed.

The highest level of the brain, the cerebral cortex, is no doubt concerned with feeding responses, but there are few experimental data relating to this subject, with the exception of certain psychological studies on animals, especially primates. Pribram and Bagshaw's observations suggest that the cortex is necessary for recognition of objects suitable for food, and with feeding responses as affected by social environment.[19] Since the work of Goltz it has been known that decorticate animals are restless at feeding time, and quiet after they are fed.[20] This observation minimizes the importance of cortical mechanisms in feeding. Neurological studies upon infants born with a cerebral lesion, or an imperfectly developed brain, also provide evidence on this point. An infant may nurse well although the cerebral cortex is grossly abnormal. Even in normal babies the regulation of feeding may not at first require the highest levels of the nervous system. Later, when habits and customs become more important, the cerebral cortex is doubtless more intimately concerned.

The second part of this paper is devoted to the many similarities between regulation of feeding and regulation of respiration—that is, pulmonary ventilation. Both feeding and breathing are periodic and rhythmic phenomena, subjected to reflex control, with integration and possible "motivation" from the brainstem. Moreover, all levels of the nervous system, including the cerebral cortex, take part in their regulation. The quantities regulated—minute volume and food intake, respectively—are in each case the product of a frequency multiplied by a quantity. Respiratory minute volume is the product of tidal air multiplied by respiratory rate, while food intake is the product of frequency of feeding and size of the meals. In the brainstem the inspiratory and appetite centers are analogous, while the expiratory and satiety mechanisms appear to be similar. Both regulations have their central mechanisms of control, yet they are each affected by specific reflexes from particular organs. For example, reflexes from chemoceptors and from stretch receptors in the respiratory tract are important in respiration, and the reflexes beginning with olfaction and taste, together with those from receptors within the wall of the digestive system, take part in the control of feeding. (The inhibition of feeding when the stomach is distended may be analogous to the inhibition of inspiration when the lungs are distended, the Hering-Breuer reflex.) And finally, both of these regulations are associated with painful sensations which are designated as hunger, for example, air hunger. It is necessary to point out that the study of pulmonary ventilation has gone forward with almost no attention to air hunger. As painful and as

dramatic as this sensation may be, it does not appear in the present-day schemes of the nature of control of respiration. Possibly its function is limited to periods of exceptional respiratory need. Whether gastric hunger is similar in its significance is not known. It has not provided a basis for any satisfactory theory of regulation of food intake, and I am inclined to the opinion that, under most conditions in adult human subjects, hunger is not very important in feeding behavior. In babies, by contrast, it may be a most critical factor, because it helps to assure a nearly helpless infant of the attention of the mother when food is needed.

In the control of pulmonary ventilation the respiratory minute volume is regulated by way of the arterial tension of carbon dioxide, the pH of arterial blood, and, apparently under unusual circumstances, by the oxygen tension. Students are often surprised to learn that a process so essential to life as is the supply of oxygen should be regulated, almost incidentally as it were, through control of carbon dioxide export. A similar situation may exist in feeding mechanisms; although the end result of regulation is the control of energy intake, animals apparently have no mechanism capable of measuring energy as such. They probably do not meter the calories ingested, but attain an energy balance rather indirectly through reactions that are related to or proportional to the energy need.

This question, what variable or what reaction is the basis of the regulation of feeding, is an important one and one of considerable interest. The third and final part of this paper is a discussion of one particular hypothesis relating to this subject. In 1931, Strang and McClugage suggested that the principal factor in satiety is the stress imposed upon the body by the extra heat produced in assimilating food.[4] The extra heat is known, of course, as the specific dynamic action (S.D.A.), and it is released in reactions where foodstuffs are interconverted and prepared for oxidation by the tissues. The S.D.A. of protein is highest because prior to oxidation it must undergo oxidative deamination, with synthesis of urea, as well as other changes peculiar to the several amino acids. The S.D.A. of carbohydrate is less, and fat least, but there is some uncertainty about these two, possibly because earlier investigators did not realize that the composition of the diet previously fed to the animal, and the length of the fasting period before the experiment, will alter the metabolic pathways for carbohydrate. Strang and McClugage carefully measured the specific metabolic effect of a mixed meal given to normal, obese, and thin human subjects; their figures illustrate well the abruptness of the increase and its magnitude.

Some years later, in 1936, Booth and Strang attempted to measure the thermal stress caused by eating, and for this purpose measured the temperature of selected regions of the skin.[5] They found that there is a rise in skin

temperature after a meal is eaten, and they interpreted this rise as being a mechanism for getting rid of some of the extra heat. The authors concluded that the cutaneous vasodilatation and the onset of satiety were interrelated, as they stated in these words: (pp. 542-3) "In these experiments the attainment of satiety, as evidenced by the cessation of eating, was also marked by a sensation of warmth in most cases and by the appearance of gross perspiration in some. These phenomena emphasize the importance of the production and dissipation of heat in the response of the body to the ingestion of food. In studies of the specific dynamic action of food, Strang and McClugage (*loc. cit.*) emphasized the physiologic load thrown on the body in normal persons by the rapid change in the production of heat resulting from the ingestion of food. One aspect of the physiologic dissipation of the extra heat is described by the observations on the temperature of the skin. . . . Certainly in persons of normal weight the inability to continue eating coincided with a rise of 0.9° C. in only twenty-two minutes."

Booth and Strang tested this hypothesis further by attempting to correlate the onset of satiety with the rate of change of skin temperature. Their data show such a correlation, but they have not been confirmed so far as I can learn; they have not, however, been contradicted. Other attempts at testing the hypothesis have been made in our laboratory, and they have without exception yielded data in agreement with the opinion of Booth and Strang. The first of our attempts was the measurement of food intake at different environmental temperatures.[6] The purpose of the study was not to confirm what was already well known—that food intake is higher in a cold than in a warm environment. It was, rather, to measure food intake when animals were exposed to an environmental temperature where they could not accept any additional heat stress and continue to maintain a normal temperature. In these particular animals under the conditions of this particular laboratory, the critical level was near 94° F. At this temperature they had a slight fever, and ate practically nothing (Fig. 1). May I say that the temperature of 94° F. is of no special significance; the temperature needed to produce this result would, among other factors, depend upon whether or not the animals were acclimatized to heat. From man and certain other animals a higher temperature would be needed to attain the same result, since man is better able to defend himself against heat stress. The important point seems to be that when the temperature of the central mechanism is brought up to the level where fever is about to occur, satiety is complete. This is analogous to apnea following hyperventilation.

Our second attempt to test the hypothesis began with the idea that the satiety value of food should be correlated with its over-all specific dynamic action. Thus, a diet high in protein content should be accepted in smaller

amount than a diet high in fat. The experiments were done by varying first the fat, then the carbohydrate, and then the protein concentration. The results were in agreement with the hypothesis.[28] In certain of the experiments the animals took in three times their usual caloric intake; this suggests that their regulation is not concerned with calories per se (Table 2). In other experiments where the density of the diet was altered, they took either larger or smaller quantities, indicating that bulk is not the most important factor. Strominger studied in this way the importance of fat, carbohydrate, and protein concentration, of bulk, caloric density, and the concentration of accessory food factors. Of all of the factors which he studied, the only one that he found to be eaten by the animals in a reasonably constant amount was the calculated specific dynamic action of the several diets. Other investigators have noted that when the central regulator in the hypothalamus is injured so as to induce hyperphagia, the disturbance is most obvious when the animals are fed a high-fat diet, and either inconspicuous or not evident when they are fed a diet high in protein.[29] Fenton and his associates have found that mice with a congenital deficiency of regulation of food intake also show this phenomenon, and become obese most rapidly when the diet is high in fat.[15] Mickelsen, *et al.*, have similar data.[15] All of these experiments

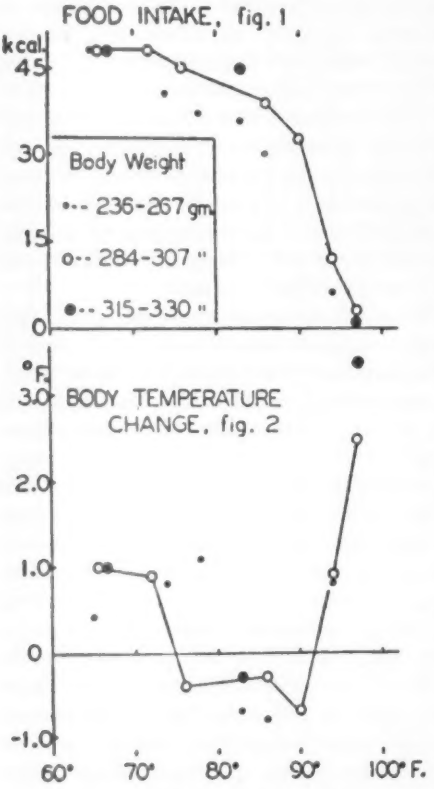


FIG. 1. Relationship between food intake and environmental temperature (upper graph) and body temperature change and environmental temperature (lower graph) in rats exposed to selected temperatures for 18 hours. Each point represents a group of 5 or 6 rats. (From J. R. Brobeck, Yale J. Biol. Med., 1948, *20*, 545.)

taken together suggest that fat is lowest in satiety value and protein highest; and when the hypothalamic regulator is not functioning properly, the low satiety value of fat becomes apparent through the abnormal weight gain of the affected animals.

The hypothesis of Booth and Strang has been utilized by members of our laboratory as an explanation of a variety of phenomena related to feeding, and since our writing on this topic has apparently not been as clear as it should have been, I hope to be able to present the subject in a straightforward manner in this discussion. First of all, the basis for the hypothesis

TABLE 2. SPECIFIC DYNAMIC ACTION OF FOOD EATEN IN 18 HOURS WHEN LARD WAS ADDED TO DIET.

(From J. L. Strominger and J. R. Brobeck, Yale J. Biol. Med., 1953, *25*, 383.)

May 2, 1946—Sprague-Dawley Males (300-325 gm.)

% Lard added to diet	*Total caloric intake*	*Partition of caloric intake*			*Calculated SDA, kcal.*			*Total SDA, kcal.*
		Protein	*CH_2O*	*Fat*	*Protein*	*CH_2O*	*Fat*	
0	54.8	13.2	36.7	4.9	4.0	1.8	0.2	6.0
14	66.4	11.0	30.9	24.5	3.3	1.5	1.0	5.8
43	90.0	7.2	19.8	63.0	2.2	1.0	2.6	5.8
57	116.3	5.8	17.5	93.0	1.7	0.9	3.8	6.4
86	110.0	1.6	4.4	104.0	0.5	0.2	4.3	5.0
100	104.6	0.0	0.0	104.6	0.0	0.0	4.3	4.3

is not the observation that animals eat less in warm environments. The true bases for the hypothesis are three in number, as follows: (i) Following eating there is a rise in heat production; this was observed by Rubner and has been confirmed countless times. (ii) Following feeding there is a slight rise in central temperature and a greater rise in skin temperature; Booth and Strang described this,[5] as did Burton and Merlin,[8] Sheard and his associates,[21] and many others. (iii) The hypothalamus contains the cells that respond to an increase in central temperature; Barbour's work suggested this more than forty years ago,[3] while the more precise experiments of Magoun and his associates identified the responsive area, at least in cats.[14] From these three types of evidence, confirmed repeatedly, it seems obvious that food is a thermal stress that acts upon the hypothalamus to bring about cutaneous vasodilatation. Furthermore, I believe that no one would seriously question a statement that food has some kind of effect upon the hypothalamus which brings about inhibition of further eating and the objective evidences of satiety. The only element of uncertainty in the

hypothesis is that the thermal effect, known to act upon the hypothalamus, is, in fact, the essential factor in satiety. The experiments where food intake was measured at different temperatures, and where the composition of the diet was altered, as described above, appear to confirm this particular step in the hypothesis.

One other subject of confusion must be mentioned. The papers from our laboratory have not stated that the Booth and Strang hypothesis is a complete explanation and the only possible mechanism for regulating food intake. There are most certainly other important factors. How some of them interact may be suggested as follows: When food is taken, sensory responses from the mouth, pharynx, esophagus, and stomach all signal to the nervous system that eating is in progress.[9] The amount of food taken may be limited first of all by this sensory input, as the depth of respiration is affected by the Hering-Breuer reflexes. Within a few minutes following eating, however, the rate of heat production begins to increase. This extra heat is produced centrally, that is, within the body itself, and it acts primarily upon the receptors of the hypothalamic or pre-optic regions. The central heating effect causes peripheral vasodilatation and, according to the hypothesis, inhibition of feeding. It apparently inhibits other mechanisms as well, those affecting posture, willingness to exercise, the excitability of spinal reflexes, and ability to stay awake. When the heat production has increased by a certain amount, or either the central or the mean body temperature has risen to a critical level (this point is undecided), the animal is not inclined to eat. Yet within a few hours the heat production falls again and with it the mean temperature, and then eating behavior is possible once more. As a tentative generalization, one may say that feeding is inhibited when heat content of the body is rising, and facilitated when heat content is falling.

As I have already mentioned, the specific dynamic action cannot be the only factor in this regulation. But it will account for so many different phenomena that one is tempted to ask whether it may account for still others. I have mentioned, for example, that if heat content of the body is already increased by exposure to a warm environment, a small amount of food will provide satiety, or intervals between meals will be longer. In a similar way, if heat content is rising because of muscular exercise, feeding will be inhibited. That exercise takes away hunger seems to be known universally. Let the exercise stop, however, with a resulting fall in heat content, and feeding is almost sure to follow. If the complications of undue fatigue, dehydration, etc., are avoided, the amount of food taken may be proportional to the rate of change of heat content when the exercise is finished.

In this same way the S.D.A. hypothesis is able to explain a phenomenon that has troubled nearly every serious student of the regulation of food intake. Many theories have rested upon the availability of some particular foodstuff or metabolite, but they have all had difficulty in explaining why at breakfast, following the period of greatest depletion, the appetite and food intake are less than at the other meals. The S.D.A. hypothesis accounts for this without difficulty. Upon arising in the morning the heat content of the body begins to increase because of the muscular activity of the waking state. Since this heat, like the S.D.A., is released within the body it affects the hypothalamus directly; it is natural that it serves as a substitute for the heat of metabolism of food. The man who enjoys his breakfast, according to the hypothesis, is the one who has breakfast in bed! Or, like a farmer or lumberjack, one who first works at his morning chores, and then sits down to eat while he is cooling off from his early exercise. I suppose that the ideal conditions for eating are an empty stomach in a body where the mean temperature is either a little low, or where it is falling rapidly.

Finally, the hypothesis may give some light upon the relationship of blood sugar levels and appetite. The data not controversial can be summarized by stating that a low level of sugar in the arterial blood increases feeding, while a hyperglycemia in an otherwise normal subject does not necessarily limit food intake. A low blood sugar level, for example, insulin hypoglycemia, very early induces signs of a heat deficit; there are both pilo-erection and shivering. (Later, of course, these are followed by other and more obvious signs of automatic activity.) No doubt the early hypoglycemia tends to diminish the resting heat production, and the body responds by conserving heat and shivering. A desire for food would naturally go with these. Hyperglycemia, on the other hand, probably has no effect upon the heat content of the animal; consequently, it should have little effect upon appetite.

REFERENCES

1. Anand, B. K. and Brobeck, J. R.: Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med., 1951, *24,* 123-140.
2. Anand, B. K., Dua, S., and Schoenberg, K.: Hypothalamic control of food intake in cats and monkeys. J. Physiol. (Lond.), 1955, *127,* 143-152.
3. Barbour, H. G.: Die Wirkung unmittelbarere Erwärmung und Abkühlung der Wärmezentra auf die Körpertemperatur. Arch. exp. Path. Pharmak., 1912, *70,* 1-26.
4. Bazett, H. C. and Penfield, W. G.: A study of the Sherrington decerebrate animal, in the chronic as well as the acute condition. Brain, 1922, *45,* 1-81.
5. Booth, G. and Strang, J. M.: Changes in temperature of the skin following the ingestion of food. Arch. intern. Med., 1936, *57,* 533-543.
6. Brobeck, J. R.: Food intake as a mechanism of temperature regulation. Yale J. Biol. Med., 1948, *20,* 545-552.

7. Brügger, M.: Fresstrieb als hypothalamisches Symptom. Helv. physiol. pharmacal. Acta, 1943, *1,* 183-198.
8. Burton, A. C. and Murlin, J. R.: Human calorimetry. III. Temperature distribution, blood flow and heat storage in the body in basal condition and after ingestion of food. J. Nutr. 1935, *9,* 281-300.
9. Janowitz, H. D. and Grossman, M. I.: Some factors affecting the food intake of normal dogs and dogs with esophagostomy and gastric fistula. Amer. J. Physiol., 1949, *159,* 143-148.
10. Kennedy, G. C.: The role of depot fat in the hypothalamic control of food intake in the rat. Proc. roy. Soc. B, 1953, *140,* 578-592.
11. Larsson, S.: On the hypothalamic organisation of the nervous mechanism regulating food intake. Acta physiol. scand., 1954, *32,* supplementum 115, 63 pp.
12. Lundbaek, K. and Stevenson, J. A. F.: Reduced carbohydrate intake after fat feeding in normal rats and rats with hypothalamic hyperphagia. Amer. J. Physiol., 1947, *151,* 530-537.
13. Lyon, J. B., Jr., Dowling, Marian T., and Fenton, P. F.: Studies on obesity. II. Food intake and oxygen consumption. J. Nutr., 1953, *51,* 65-70.
14. Magoun, H. W., Harrison, F., Brobeck, J. R., and Ranson, S. W.: Activation of heat loss mechanisms by local heating of the brain. J. Neurophysiol., 1938, *1,* 101-114.
15. Mickelsen, O., Takahashi, S., and Craig, C.: Experimental obesity. I. Production of obesity in rats by feeding high-fat diets. J. Nutr., 1955, *57,* 541-54.
16. Miller, F. R. and Sherrington, C. S.: Some observations on the bucco-pharyngeal stage of reflex deglutition in the cat. Quart. J. exp. Physiol., 1916, *9,* 147-186.
17. Miller, N. E., Bailey, C. J., and Stevenson, J. A. F.: Decreased "hunger" but increased food intake resulting from hypothalamic lesions. Science, 1950, *112,* 256-259.
18. Olds, J.: "Rewards" from brain stimulation in the rat. Science, 1955, *122,* 878.
19. Pribram, K. H. and Bagshaw, Muriel: Further analysis of the temporal lobe syndrome utilizing frontotemporal ablations. J. comp. Neurol., 1953, *99,* 347-375.
20. Rogers, F. T.: Contribution to the physiology of the stomach. XXXIX. The hunger mechanism of the pigeon and its relation to the central nervous system. Amer. J. Physiol., 1916, *41,* 555-570.
21. Sheard, C., Williams, M. M. D., and Horton, B. T.: Skin temperatures of the extremities under various environmental and physiological conditions. In *Temperature,* American Institute of Physics. New York, Reinhold Publishing Corp., 1941, pp. 557-570.
22. Strang, J. M. and McClugage, H. B.: The specific dynamic action of food in abnormal states of nutrition. Amer. J. med. Sci., 1931, *182,* 49-81.
23. Strominger, J. L. and Brobeck, J. R.: A mechanism of regulation of food intake. Yale J. Biol. Med., 1953, *25,* 383-390.
24. Teitelbaum, P.: Sensory control of hypothalamic hyperphagia. J. comp. physiol. Psychol., 1955, *48,* 156-163.
25. Teitelbaum, P. and Stellar, E.: Recovery from the failure to eat produced by hypothalamic lesions. Science, 1954, *120,* 894-895.

J. A. F. STEVENSON*
R. H. RIXON**

Department of Physiology, Faculty of Medicine, University of Western Ontario, London, Ontario, Canada

ENVIRONMENTAL TEMPERATURE AND DEPRIVATION OF FOOD AND WATER ON THE SPONTANEOUS ACTIVITY OF RATS†

INTRODUCTION

Deprivation of food, water, or individual nutrients usually causes an increase in the spontaneous running activity of the rat[2, 9, 10, 17, 18, 22, 27, 28, 30] although some exceptions have been reported.[2, 17, 30] The measurement of running activity has been used for investigations into motivation, for instance, as a measure of the hunger and thirst drives.[9, 10, 27] Wald and Jackson[30] considered the hyperactivity of rats during nutritional deprivation an example of mammalian emigration to relieve the nutritional pressure on the home population.

On the other hand, muscular activity is one of several means by which body temperature can be maintained through an increase in heat production.[4, 5, 7, 14] If the increased activity during starvation primarily reflects some function in the maintenance of body temperature, then there should be a relation between the environmental temperature and the degree of activity in starvation.

METHODS

Male Sprague-Dawley rats (300-400 gm.) were fed a synthetic diet (4.2 cal./gm.). In this diet approximately 70 per cent of the calories were derived from sucrose, 20 per cent from crude casein and 10 per cent from corn oil. Adequate amounts of the known necessary vitamins and salts were added to the mixture.[29] Food and water were provided *ad libitum*, except when one or the other was specifically restricted. The rats used in the acclimatization study were eight to nine weeks old at the beginning of the 28-week experiment. All other rats were 10 to 12 weeks old at the start of the experiments.

No other animals were kept in the experimental room and it was entered only for daily care and recording. The lights were automatically turned on at 8 a.m. and off at 8 p.m. The temperature of this room could be controlled to ± 1° C. at levels from 5

---

* Professor and Head, Department of Physiology.

** Research Officer, Biology Branch, Atomic Energy of Canada Limited.

† This work was supported by grant 8865-02 from the Defence Research Board of Canada.

Received for publication November 28, 1956.

to 35° C. The relative humidity was kept at 50 per cent, except at 10° C. and below when it could not be maintained and rose to about 90 per cent.

The animals lived in individual activity cages unless otherwise specified; some control studies were made in ordinary individual cages (7 x 9 x 6 inches). The activity cage consisted of a revolving drum, 14 inches in diameter, and a small living cage separable from the drum by a sliding door.* This door was never closed during these experiments. A Veedor counter recorded each complete revolution of the drum. Twelve of these cages were arranged in three rows on a specially constructed rack. As noted by Reed[17] the animals required about 10 days to adjust to the activity cage and showed a consistent level of activity following this period.

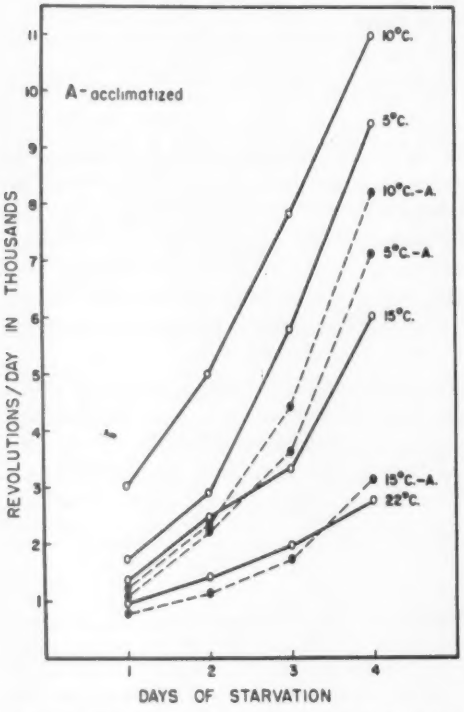


FIG. 1. The increases in spontaneous activity which occurred during four days of starvation at various environmental temperatures.

Skin and colonic temperatures were recorded by thermocouples.† The representative skin temperature was taken as the average of readings from three areas, the back, about one inch from the base of the tail, and the "thighs" of each hind leg. Each of these areas had been shaved. The colonic temperatures were obtained by inserting a rectal thermocouple 2 to 2½ inches past the anal orifice.

RESULTS

Figure 1 shows the increase in activity which occurred at environmental temperatures from 5° C. to 22° C. during four days of starvation. The running activity progressively increased up to the fourth day of food deprivation. The rate of increase was dependent on the surrounding temperature and was greatest in the cold environments. Curves at higher environmental temperatures (27°, 30°, 33° C.) are not shown since they were similar to,

* From Geo. H. Wahmann Mfg. Co., Baltimore, U.S.A.
† Electric thermometer manufactured by Light Laboratories, Brighton, England.

but shallower than, that at 22° C. Since the limit of survival for those animals in the cold was about four days, the fourth day was chosen for comparison of the activity at all environmental temperatures.

Figure 2 shows the spontaneous running activity of rats during the fed state and on the fourth day of starvation at environmental temperatures ranging from 5° to 33° C. In the fed state, the activity was about 1000 rev./day at environmental temperatures from 15° to 27° C., decreasing to 450 at 33° C. and to 700 at 5° C. This decrease in warm and cool environment has been noted by Reed.[17]

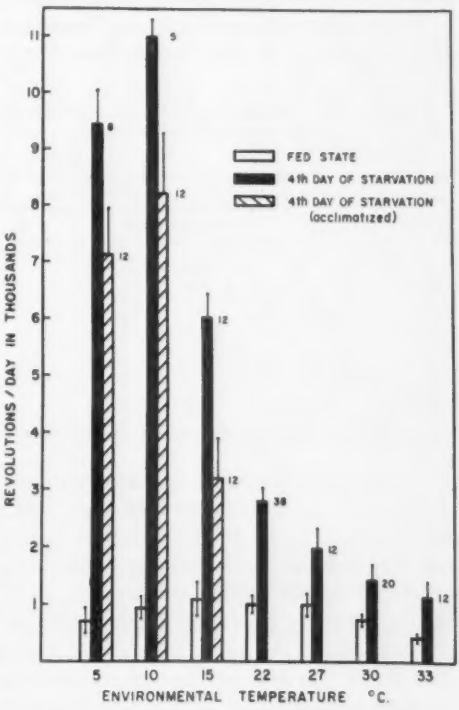


FIG. 2. The spontaneous running activity of rats in the fed state and on the fourth day of starvation at various environmental temperatures. Vertical lines represent the standard error.

During starvation there were increases in spontaneous activity which varied considerably with the environmental temperature. At 22° C. the average activity had risen to 2800 rev./day by the fourth day. In warmer environments the increase was less, reaching 1,150 at 33° C. At temperatures below 22° C. the activity showed very marked increases, rising to 6,000 at 15° C., 11,000 at 10° C., but only to 9,450 at 5° C. These animals at 15°, 10°, and 5° C. were groups of rats (originally 12 in each group) not fully acclimatized to the cold. They were adjusted to the activity cages at 22° C. and then exposed to one of the lower temperatures for 10 to 15 days before starvation.

Figure 2 also contains the results from 12 animals that were exposed to all the environmental temperatures studied. These animals were taken gradually from 33° to 5° C. over a period of seven months, with intervals

of adaptation, starvation, and recuperation at each of the designated temperatures. The time spent at each temperature was about four weeks. In view of the exposure to gradually decreasing temperatures and the time spent at each temperature, it is probable that these rats were fairly well acclimatized to the cold.[6, 23, 24, 25, 26] No acclimatization was evident in the activity of rats at temperatures of 22° C. and above.

The acclimatized rats displayed a lower average activity in starvation at 15°, 10°, and 5° C. than the corresponding nonacclimatized groups. None

TABLE 1. BODY WEIGHT LOSS DURING FOUR DAYS OF STARVATION

Env. temp. 0° C.	*Body weight loss (gm.)*		
	Activity cage		*Ordinary cage*
	Nonacclimatized	*Acclimatized*	
5	109	99	110
10	111	100	
15	72	64	
22	63		52
27	48		
30	48		
33	42		41

of the acclimatized animals died at any temperature during their four days of starvation, whereas in the nonacclimatized groups, seven died at 10° C. and four at 5° C. At 10° C. several of these animals were found prostrate and very cold on the fourth day of starvation after having run close to 13,000 revolutions during the previous 24 hours. Two of these animals were successfully revived by placing them in a warmer environment.

Table 1 shows the loss of body weight during the four days of starvation. Both the weight loss and the activity increased as the environmental temperature dropped. Rats of comparable weight were starved in ordinary individual cages at various environmental temperatures; these animals showed losses of body weight in four days similar to those of animals in the activity cages. At 22° C., the average length of survival in the ordinary cage was about nine days and in the activity cage about seven days. In the cold (5° C.) the survival was close to four days[39] after starvation in either type of cage.

Figure 3 demonstrates the effects of partial food restriction and complete water deprivation on the spontaneous activity and body weight loss when rats were given one-third (7 gm./day) of their voluntary food intake at 22° C. The daily activity gradually increased over a period of 17 days, when

maximal activity appeared to have been reached at slightly under 2,000 rev./day. This is lower than that observed in the completely starved rat. Refeeding resulted in an abrupt return to, or slightly below, the initial level of activity. This fall in activity on return of food was characteristic of ani-

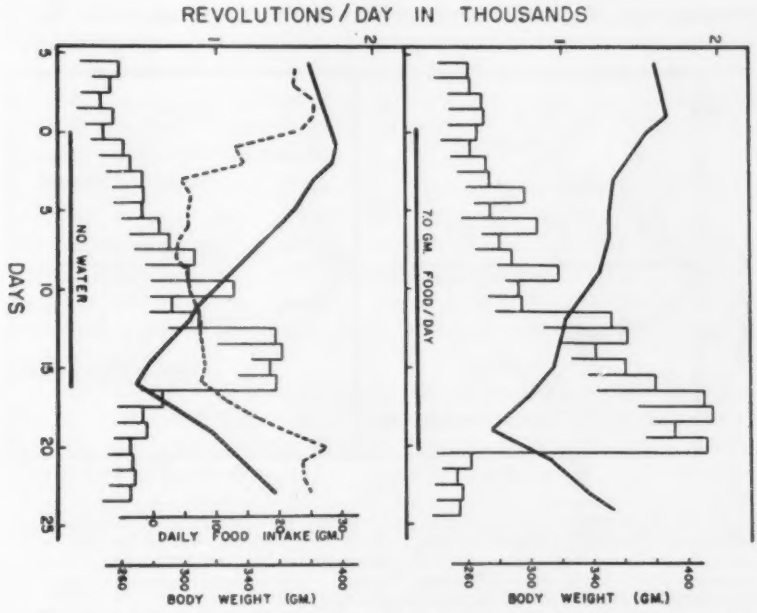


FIG. 3. The daily spontaneous activity, body weight loss, and food intake during (a) partial food and (b) complete water deprivation at an environmental temperature of 22° C. Eight rats were used in each study.

mals at all temperatures. Finger[9] also has reported a depression of activity following restoration of food.

Complete water restriction produced a similar picture. The activity increased gradually over a period of 13 days to form a plateau at close to 1,500 rev./day. The drop in activity on the first day after the return of water has also been observed by Finger and Reid,[10] but the depression below pre-thirst levels did not appear in our animals. For most of the period of water lack the food intake was about 7 gm./day but during maximal activity it was 8 gm./day.[cf.10] This food intake may account for the slightly lower maximal activity of these animals compared to the partial food-restricted group.

Preliminary experiments have been done on the skin and colonic temperatures of rats during starvation at 22° C. Figure 4 shows the temperature changes of rats in canvas slings, to minimize muscular activity, and in those allowed to move freely in ordinary individual cages (not activity cages). The animals starved in the slings showed a decline in both skin and colonic temperatures. The largest decrease was in the skin temperature. These

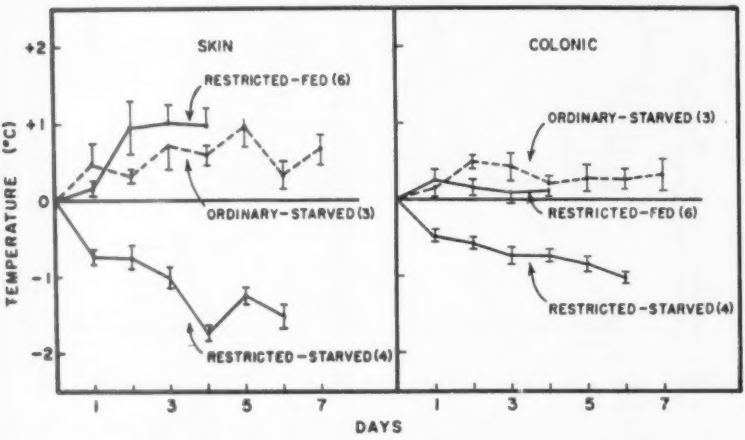


FIG. 4. The changes in skin and colonic temperatures of rats starved in canvas slings (Restricted-Starved), fed while in slings (Restricted-Fed) and starved but allowed to move freely in ordinary single cages (Ordinary-Starved). Vertical bars represent the standard error of the mean difference. Numbers in parenthesis represent number of animals. The environmental temperature was 22° C.

decreases did not appear in rats which were fed during a four-day period in the slings. Rats starved but allowed to move freely maintained their skin and colonic temperatures for at least the first six days of complete food deprivation.

DISCUSSION

Starvation causes an increase in the spontaneous activity of the rat. The inverse relation which we have observed between the level of the environmental temperature and the amount of activity supports the hypothesis that this increase in activity is related to the maintenance of body temperature in starvation. This hypothesis is further supported by our preliminary observations that the body temperature tends to fall in starvation when activity is restricted, but not when it is permitted.

A relation between activity and metabolic rate has long been considered[11,12,13,16,17,20] and it is well known that the metabolic rate increases as the environmental temperature falls.[1, 6, 19, 22, 24, 26] Thus the great increase in activity upon starvation in the cold might be considered a reflection of the raised metabolic rate. The increase in metabolism on exposure to cold, however, occurs even in the fed state and in this state there is no increase in activity during exposure to cold. On the other hand, in a temperate environment, the metabolic rate usually falls as starvation progresses, but in the cold there is no fall in metabolic rate on starvation.[19] Thus, there seems to be little, if any, direct relation between the level of activity and the level of the basal metabolic rate in starvation.

If food is available *ad libitum,* rats maintain their colonic temperature, and may even increase it slightly, when exposed to a cold environment.[1, 23] Starvation in man may cause temperature to fall to 35° C. and a diminished cutaneous circulation is suggested by pallor of the skin.[15] The present study indicates that, in the rat, starvation causes a fall in the core and peripheral temperature of the rat when activity is severely restricted, but not when activity is possible.

It appears from the similarity of the weight losses in the two cages that starvation increases the activity of rats in ordinary individual cages or in activity cages equally. Our methods of observation did not measure activity in the form of shivering and it is possible that the animals in the individual cages achieved a part of their increased muscular activity in this manner. Increased shivering may have decreased the running of acclimatized and nonacclimatized rats at 5° C. compared to that at 10° C.[8]

Bargeton *et al.*[1] have found that the heat production of acclimatized rats is less than that of nonacclimatized rats in a cold environment. From this it may be inferred that acclimatization is associated with a reduced loss of heat. And it is the acclimatized animals which show a smaller increase in activity on starvation in the cold. The improved conservation of heat has perhaps reduced the demand for its production to maintain body temperature.

The ability of the body to conserve heat is dependent on its effective insulation and this is increased in various ways in the acclimatized animal. Starvation undoubtedly causes a deterioration in this insulation; e.g. through catabolism of the subcutaneous fat. With the progress of starvation, the ability to conserve heat may thus steadily decline and lead to an increasing demand for the production of heat to maintain body temperature. This increasing demand could be supplied, in part at least, by a steady increase in muscular activity.

Brobeck[6] has supported the hypothesis that regulation of food intake is related to the regulation of body temperature. He and his colleagues[7] have also observed that a decline in body temperature precedes the rise in running activity that occurs with estrus and that the low activity in pseudopregnancy is associated with a high body temperature. Ryan[21] has recently shown that dinitrophenol causes a marked increase in the body temperature of dogs and it is known[11, 12] that this drug, which increases the oxygen consumption and presumably the heat production, reduces activity.

Muscular activity appears to be closely related to the maintenance of body temperature, and to be interrelated with food intake in this function. The increase in activity on deprivation of water would not, at first, appear to support this conclusion, but even in this circumstance the degree of activity was related to the amount of food eaten.

SUMMARY

1. The running activity of rats increased in starvation. This increase in activity was inversely related to the environmental temperature between 33° and 10° C. and varied from 2 to 14 times that in the fed state. The activity at 5° C., however, was lower than that at 10° C.

2. Starvation did not produce as great an increase in the activity of acclimatized as in nonacclimatized rats at temperatures from 5° to 15° C.

3. The weight loss during four days of starvation in activity or in ordinary cages was similar and increased as the environmental temperature decreased.

4. Rats restricted to one-third (7.0 gm.) of their voluntary daily food intake, or completely deprived of water, increased their activity gradually and reached a maximum activity in 17 and 13 days, respectively. This activity was less than that after four days of starvation. The activity of the water deprived group was slightly less than that of the partial food-restricted animals but the food intake of the former was 8.0 gm. during maximal activity. Both groups showed a sudden drop to, or below, their initial level of activity on return of food or water *ad libitum*. This also occurred after complete starvation.

5. The rectal and skin temperatures decreased during starvation when activity was severely restricted, but not when the animals were fed and restricted simultaneously or starved with freedom of movement.

It is suggested that the increased activity during starvation is related to the maintenance of body temperature and is interrelated with food intake in this function.

ACKNOWLEDGMENTS

We wish to thank Mr. M. Barnes and Miss L. Myiata for their technical assistance. Dr. Ruth Wolfe of Hoffmann-LaRoche kindly supplied the vitamins used in the synthetic diet.

REFERENCES

1. Bargeton, D., Eon, M., Krumm-Heller, C., Libermann, C., and Masson, J.: Influence de l'adaptation sur les réactions thermoregulatrices au froid chez le rat. J. Physiol. (Paris), 1954, *46,* 845.
2. Bevan, W., Jr., Lewis, G. T., Bloom, W. L., and Abess, A. T.: Spontaneous activity in rats fed an amino acid-deficient diet. Amer. J. Physiol., 1950, *163,* 104.
3. Bloomfield, A. L. and Tainter, M. L.: The effect of Vitamin B deprivation on spontaneous activity of the rat. J. Lab. clin. Med., 1942-43, *28,* 1680.
4. Brobeck, J. R.: Effects of variations in activity, food intake and environmental temperature on weight gain in the albino rat. Amer. J. Physiol., 1945, *143,* 1.
5. Brobeck, J. R.: Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol. Rev., 1946, *26,* 541.
6. Brobeck, J. R.: Food intake as a mechanism of temperature regulation. Yale J. Biol. Med., 1948, *20,* 545.
7. Brobeck, J. R., Wheatland, M., and Strominger, J. L.: Variations in regulation of energy exchange association with estrus, diestrus, and pseudopregnancy in rats. Endocrinology, 1947, *40,* 65.
8. Burton, A. C. and Edholm, O. G.: *Man in a cold environment.* London, Edward Arnold Ltd., 1955. 273 pp.
9. Finger, F. W.: The effect of food deprivation and subsequent satiation upon general activity in the rat. J. comp. physiol. Psychol., 1951, *44,* 557.
10. Finger, F. W. and Reid, L. S.: The effect of water deprivation and subsequent satiation upon general activity in the rat. J. comp. physiol. Psychol., 1952, *45,* 368.
11. Hall, V. E. and Lindsay, M.: The effect of dinitrophenol on the spontaneous activity of the rat. J. Pharmacol., 1934, *51,* 430.
12. Hall, V. E. and Lindsay, M.: The relation of the thyroid gland to the spontaneous activity of the rat. Endocrinology, 1938, *22,* 66.
13. Hoskin, R. G.: Endocrine factors in vigor. Endocrinology, 1927, *11,* 97.
14. Houssay, B. A., Lewis, J. T., Orias, O., Braun Menendez, E., Hug, E., Fohlia, V. G., and Leloir, L. F.: *Human physiology.* 1st ed. New York, McGraw-Hill Book Company, Inc., 1951. pp. 490 *et seq.*
15. Keys, A., Brozek, J., Henschel, A., Mickelson, O., and Taylor, H. L.: *The biology of human starvation.* Rochester, Minn., University of Minnesota Press, 1950. Vol. I, pp. 612 *et seq.*
16. Lee, M. O. and Buskirk, E. F. van: The effect of thyroidectomy on spontaneous activity in the rat, with a consideration of the relation of the basal metabolism to spontaneous activity. Amer. J. Physiol., 1928, *84,* 321.
17. Reed, J. D.: Spontaneous activity of animals. Psychol. Bull., 1947, *44,* 393.
18. Richter, C. P. and Rice, K. K.: Comparison of the effects produced by fasting on gross bodily activity of wild and domesticated Norway rats. Amer. J. Physiol., 1954, *179,* 305.
19. Rixon, R. H. and Stevenson, J. A. F.: Factors influencing the survival of rats in starvation: metabolic rate and body weight loss. Amer. J. Physiol., 1957, *188,* 332.
20. Rundquist, E. A. and Bellis, C. J.: Respiratory metabolism of active and inactive rats. Amer. J. Physiol., 1933, *106,* 670.

21. Ryan, E. A.: Studies on the effect of 2:4 dinitrophenol of liverless and diabetic dogs. Amer. J. Physiol., 1951, *167,* 224.
22. Schmidt-Nielsen, K. and Schmidt-Nielsen, B.: Water metabolism of desert mammals. Physiol. Rev., 1952, *32,* 146.
23. Sellers, E. A., Reichman, S., and Thomas, N.: Acclimatization to cold: natural and artificial. Amer. J. Physiol., 1951, *167,* 644.
24. Sellers, E. A., Reichman, S., Thomas, N., and You, S. S.: Acclimatization to cold in rats: metabolic rates. Amer. J. Physiol., 1951, *167,* 651.
25. Sellers, E. A. and You, S. S.: Role of thyroid in metabolic responses to a cold environment. Amer. J. Physiol., 1950, *163,* 81.
26. Sellers, E. A., You, S. S., and Thomas, N.: Acclimatization and survival of rats in the cold: effect of clipping, of adrenalectomy and of thyroidectomy. Amer. J. Physiol., 1951, *165,* 481.
27. Siegel, P. S. and Steinberg, M.: Activity level as a function of hunger. J. comp. physiol. Psychol., 1949, *42,* 413.
28. Souto-Maior, M. G. and Foglia, V. G.: Ia actividad espontanea de la rata diabetica. Rev. Soc. Argent. Biol., 1944, *20,* 55.
29. Stevenson, J. A. F.: *Cold injury.* M. I. Ferrer, Ed. Transactions of the Third Conference, February 22, 23, 24, and 25, 1954. New York, The Josiah Macy, Jr. Foundation, 195 pp. 165 *et seq.*
30. Wald, G. and Jackson, B.: Activity and nutritional deprivation. Nat. Acad. Sci. Proc., 1944, *30,* 255.

HENRY M. PARRISH*

Department of Public Health and the Department of Psychiatry, Yale University School of Medicine

EPIDEMIOLOGY OF SUICIDE AMONG COLLEGE STUDENTS**

INTRODUCTION

Suicide is more common among college students than many people realize. A recent survey of the causes of death of 209 Yale University students from 1920 to 1955 revealed the following most common causes of death: (i) accidents—43.8 per cent; (ii) suicide—12 per cent; (iii) heart and circulatory diseases—7.7 per cent; (iv) pneumonia—7.2 per cent; and (v) central nervous system infections—6.3 per cent.[18] Diehl and Shepard[8] made a similar study of 327 deaths among college students from nine universities during the ten-year period from 1925 to 1935. They found the following leading causes of death: (i) accidents—26.3 per cent; (ii) heart and circulatory diseases—10.1 per cent; and (iii) suicide—8.0 per cent. One may conclude from these two studies that suicides account for 8 to 12 per cent of all deaths among college students.

It has been estimated that more than 20,000 people commit suicide in the United States each year.[18] Dublin[5] reported the suicide rate for the United States in 1930, 15.6 per 100,000 living, occupies a middle position among the rates of countries for which the records are available. Collins *et al.*[3] found suicide the fifth most common cause of death in the white population of the United States, for the age group from 15-24, for 1950. A review of the available literature failed to provide any reliable statistics on the incidence of suicide among college students. It is commonly believed that the incidence of suicide is higher among college students than among the non-college population in the same age group. This opinion was recently expressed by Mr. T. R. Henn, Senior Tutor of St. Catherine's College, Cambridge, England when he addressed the World Conference on Medical Education. Mr. Henn stated:

---

* Fellow in Public Health and Assistant Physician, Department of University Health.

** This paper is based on a study in partial fulfilment of the requirements for the degree of Master of Pubic Health.

Received for publication October 24, 1956.

Although there are no accurate statistics, the incidence of suicide at the two older universities (Oxford and Cambridge) is higher than at the other universities in Great Britain. The incidence was much higher in students than in comparable groups of non-students.[9]

It is obvious that statements of this nature, without facts to support them, merely express the opinion of one individual. In a letter to the editor of *The London Times,* Mr. S. R. Dennison, Senior Tutor of Caius College, Cambridge, replied to Mr. Henn's remarks:

The Senior Tutor of St. Catherine's College is reported to have informed the World Conference on Medical Education that the incidence of suicides among students is higher at Oxford and Cambridge than at other universities, although admitting that no accurate statistics exist and doubting whether they could be made available. I suggest that there are no statistics whatever of the kind needed to make proper comparisons between universities, and with other groups in the non-university population. Until we know some of the simple facts, we should be well advised to avoid diagnosing, and prescribing for, a problem which so far has not been shown to exist.[8]

PURPOSE

The purpose of this report was to collect some of the simple facts which appear to be lacking about suicides among college students, to compare the incidence of suicide among college students with that of the noncollege population, and to determine some of the epidemiological factors related to suicide in this group. There are many theories concerning the psychopathogenesis of suicide and many environmental factors that seem to precipitate the act in an individual; therefore, an epidemiological approach was selected for this study. Gordon[8] emphasized the "biologic gradient" of disease in living organisms—the disease may result in complete recovery, a complication, or death. Previous studies failed to point out a specific cause for suicide, but suggested that suicide is a complex interaction of personality, environment, and culture. The epidemiologist would use slightly different terms—the host, the agent, and the environment. What are these factors in suicide among college students?

MATERIALS

The suicides reported in this study were taken from the files of the Department of Mental Hygiene and Psychiatry, the Department of University Health, and the Alumni Records Office of Yale University. A *student suicide* was defined to include students who took their lives during the academic year or summer vacation, and any student who withdrew from college or was given a psychiatric leave of absence and committed suicide within one year of his separation.

Each suicide was confirmed by a death certificate from the Bureau of Vital Statistics in the town where death occurred. Valuable information was obtained from the individual records of the deceased in the Department of Alumni Records. In most instances these records contained the personal history of the student and his family, the date the student withdrew from college (because of death or a psychiatric leave of absence), a death certificate, and newspaper clippings describing the circumstances of the suicide. Medical records from the Department of University Health provided the student's past medical history, his family medical history, and any diseases he had while in college. The psychiatric record of any student seen in the Department of Mental Hygiene was studied and the diagnosis recorded. The Registrar's Office supplied the student's academic record, and the class yearbooks described his extracurricular activities.

INCIDENCE AND PREVALENCE

Suicide accounted for 25, or 12 per cent, of 209 student deaths among Yale University students from 1920-1955. It was second only to accidents as a leading cause of death in the student population. Each year approximately 20-30 students are seen by the Department of Mental Hygiene who have serious suicidal thoughts. The present study was confined to actual deaths or successful suicidal attempts.

The crude death rate of Yale students was lower than the crude death rate of a comparable noncollege population. Of the 209 students who died during the 35-year period studied, 95 per cent were white males, and 86 per cent were in the 15-24-year age group. This being the case, Yale suicides were compared with suicides in the general population for the year 1950 (white males 15-24 years of age).[1] There was only one death at Yale in 1950, consequently the suicides from 1945-1950 were used to calculate the specific-cause-of-death rate. This procedure seemed legitimate since the student population and the national economy remained stable during that period. There were 5.1 suicides expected, if the national rates prevailed, and 7.0 suicides observed. The national suicide death rate was 6.6 per 100,000 for white males 15-24 years of age, and the Yale student suicide death rate was 9.0 per 100,000. This difference was not statistically significant using the Chi-square technique.

AGE, SEX, AND CLASS

All of the suicide victims were white males except for one white female. This uneven sex distribution should not be considered unusual, for women

are admitted only to the graduate and professional schools. All of the students were native-born Americans, except for one French graduate student.

There were 15 suicides among undergraduate students, and 10 among graduate and professional school students. The age of the undergraduates ranged from 18-24 years with an average of 20.2 years. The ages of the graduate students ranged from 21-31 years with an average age of 26.4 years. There were 13 suicides in the age group from 18-21, 7 in the age

TABLE 1. DEATH RATE FROM SUICIDE AMONG YALE STUDENTS 1920-1955

Class	*Number of suicides*	*Population at risk***	*Death rate per 100,000*
Freshman*	2	32,231	6.2
Sophomore*	7	27,590	25.4
Junior*	2	27,590	7.2
Senior*	4	27,590	14.5
Medical	2	8,172	24.5
Law	1	12,614	7.9
Divinity	1	9,001	12.2
Graduate	5	25,349	19.7
Nursing	1	3,781	26.4

* Undergraduate classes.
** These figures calculated from University Catalogues of Yale University, 1920-1955.

group from 22-25, and 5 in the age group from 26-31. The lower incidence in the latter group can be accounted for by a smaller population at risk, since 86 per cent of 209 students who died from all causes were in the age group from 15-24 years. Table 1 shows the number of suicides in each class, the population at risk in each class, and the suicide death rate per 100,000 for each class. The highest rates were found in the undergraduate sophomore class, the medical classes, and the nursing classes. The lowest rate was in the undergraduate freshman class. The difference in these death rates was not statistically significant because the number of suicide cases studied was small. They do suggest that more extensive investigation might prove fruitful.

PLACE AND METHOD

Ten students committed suicide in the college community, New Haven; nine in either their dormitory or fraternity house room, and one in a local hotel. They were enrolled in school at the time of death. Eleven took their lives at home—four of them were enrolled in school at the time of death,

and seven were home on a psychiatric leave of absence or had withdrawn from school. Four students killed themselves in localities away from both school and home. They were all enrolled in school at the time.

Dublin[4] stated that about four-fifths of those who die by their own hand in the United States use one of four methods—shooting, hanging, poisoning, or asphyxiation by gas. White males use firearms as the most frequent method of suicide. In keeping with this observation, 96 per cent of Yale students died by one of these four methods. Table 2 summarizes the method of suicide selected by the students.

TABLE 2. METHOD OF SUICIDE USED BY YALE STUDENTS 1920-1955

Method	*Number*	*Per cent*
Firearms	10	40
Hanging	6	24
Asphyxiation	5	20
Poisoning	3	12
Jumped from a a high place	1	4
Total	25	100

Some of the student suicide victims seemed to show psychic determinants in the choice of their method. It is interesting that three of the ten students who used firearms were gun collectors and disposed of themselves with a favorite weapon. The three students who used poison had an intimate knowledge of chemistry. Two undergraduate chemistry students selected potassium cyanide, a classical poison. One medical student chose "Black Leaf 40," a deadly insecticide containing nicotine, in spite of the fact that sleeping pills were readily accessible to him. Possibly he realized that sedatives have a slower onset of action, and his chances of being discovered and revived were greater if he used sedatives.

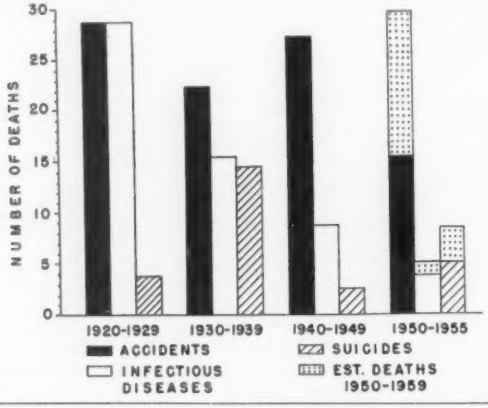
TIME RELATIONSHIPS

Studies of large numbers of suicides show an incidence peak in the spring and early summer months. The largest number of suicides found in this report were in January (five) and April (four). The number of suicides by months were: five in January, one in February, none in March, four in April, two in May, one in June, two in July, one in August, three in September, one in October, two in November, and three in December. Four of

the deaths occurred during the Christmas vacation period, six during the summer vacation period, and fifteen during the school year.

The day of the week students selected to do away with themselves were: four on Monday, two on Tuesday, four on Wednesday, nine on Thursday, one on Friday, two on Saturday, and three on Sunday. The day a student chose for suicide seemed significant in three instances—two students selected New Year's Day, and one student took his life on the birthday of a former roommate who had committed suicide two years before.

TABLE 3. DEMOGRAPHIC CHANGES OF YALE STUDENT DEATHS 1920-1955



The time of day students killed themselves was as follows: ten died from 12 midnight to 6 a.m.; four died from 6 a.m. to 12 noon; six died from 12 noon to 6 p.m.; and four died from 6 p.m. to 12 midnight. The time of death could not be found for one student. These data suggest that insomnia associated with suicidal thoughts accounts for a large number of suicides late at night and in the early morning hours.

A study of the demographic changes of Yale student deaths from 1920-1955 may be found in Table 3. Deaths from communicable diseases have declined since 1920-1929, placing accidents as the number one killer of college youths. The number of suicides rose sharply during the depression years, then dropped to a lower level during, and immediately following, World Wars I and II. In general, suicide rates increase during periods of economic stress, and decline during wars. This held true for the incidence of suicide among college students.

DIAGNOSIS

Only 11 of the 25 students who committed suicide were seen in the Department of Mental Hygiene and Psychiatry. The Department was not organized until October 3, 1925; the actual patient work began a year later. One suicide occurred in 1925 before the Department was organized. Therefore, only 45 per cent of those who took their lives received psychiatric treatment. Three students were referred to the Department by faculty members, four by physicians from the Department of University Health, one by a roommate who majored in psychology, one by a private psychiatrist who had been treating a student, one by a freshman counselor (a student), and one was a self-referral (a medical student). Of the students who were treated by the Department, the following diagnoses were made: psychotic depression—3; schizophrenia, paranoid type—1; anxiety reaction—2; acute dissociative reaction with paranoid features—1; severe character neurosis—1; chronic alcoholism—1; and psychopathic personality with homosexuality—1.

Eight of the students not seen by the Department killed themselves unexpectedly; no personality changes were noted by either faculty members or classmates. Four students who were not seen by the Department had expressed feelings of futility and depression to classmates, and, in addition, two students who showed a lack of interest in their school work, excessive absence from classes, and infraction of school regulations were known to the faculty. They were not referred for counseling.

The symptoms observed among students, in order of their frequency, were: feelings of despondency and futility; lack of interest in school work with, or without, frequent class cutting; a feeling of tenseness around people; insomnia; suicidal thoughts communicated to others; fatigue and malaise without an apparent organic cause; a feeling of inadequacy and unworthiness; excessive use of alcohol; antisocial behavior with frequent infraction of school regulations; and brooding over the death of a loved one.

It has been stated that physical illness, especially in older people, is a frequent motive for suicide. This did not prove the case among these students. Only one patient had a serious medical problem: a duodenal ulcer which was asymptomatic at the time of death.

COLLEGE ACTIVITIES

A common fallacy is that only brilliant, talented students commit suicide. Only 3 of the 25 students in this study were Phi Beta Kappa members. Of those who took their lives, 6 were excellent students, 5 were poor students, and the majority, 14, were average students. There were noticeable changes

in the academic records of 5 students the semester before their act of self-destruction. Their grades declined from 12 to 25 points with an average drop of 15 points per student. This sudden drop in grades probably reflects the anxious, agitated state of a student with lack of interest in, and inability to concentrate on, scholastic material. A sudden change in grades may provide the alert teacher or administrator with a clue that a student is emotionally disturbed.

What type of student is more prone to take his own life? Many people have the preconceived notion that the introvert—the quiet, bookworm who doesn't mix well socially—is more susceptible. Of 25 students who destroyed themselves, 10 were very active in extracurricular activities, 3 were moderately active, and 12 were not active. There were 10 fraternity men and 15 nonfraternity members. Six of the group were outstanding athletes. There were 22 single students, 2 married students with children, and 1 separated student with a child. Fourteen students came from families in which both parents were living together, 5 came from broken homes, 3 came from families in which one parent had died and the other had remarried, 2 came from families in which one parent was deceased and the other had not remarried, and 1 came from a family in which both parents were dead. Only three of the family histories were positive for mental disease. The father's occupation in 6 instances was an executive, 4 were professional men, 4 were bankers, 3 were educators, 3 worked in business offices, 2 were writers, and 1 was a farmer. Thus, 14 students came from families that were financially well-to-do.

The religious affiliation listed by the deceased were: Protestant—20, nonaffiliated—4, and Roman Catholic—1. There were no suicides among the Jewish students. One difficulty with statistics of this kind is that often the population at risk—the number of students of each denomination—is not known. Fortunately, these data are available. During the past 35 years there have been approximately 62 per cent Protestant students, 14 per cent Catholic students, 13 per cent Jewish students, and 11 per cent nonaffiliated students. Of course, these data do not relate how "deeply religious" an individual may have been. No valid conclusions can be drawn about the incidence of suicide among these different religious groups.

AREAS OF STRESS

Financial problems were an important source of environmental stress for eight students. Six students were either on scholarships or working to support themselves. One student took his life when he lost a job that would have allowed him to continue his education. A graduate student who was

teaching while working for an advanced degree became despondent when his wife became pregnant for the third time. Evidently, his teaching assignments, his studies, and his financial worries with four mouths to feed were too much for him. Another scholarship student was observed by his professor to be working too hard; this was just two weeks before he killed himself. Two students who were unable to keep up with their wealthy friends complained of insufficient funds; one of them committed suicide the day he received a letter from his mother reprimanding him for overspending and stating that if this practice did not stop he would have to withdraw from school.

Sexual adjustments were contributing difficulties for six students. Three of them had proposals for marriage rejected, and shortly afterwards found life unbearable. One student murdered the girl who turned him down, then shot himself. One medical student who had suffered a depression during college killed himself just 10 days after becoming engaged. He feared that he would become insane and ruin his marriage. One student with marital difficulties took his life soon after becoming estranged towards his wife. Another student, an overt homosexual, was overcome by feelings of guilt and anxiety.

Moss and Hamilton[11] recently called attention to "the death trend" as a significant feature of suicide patients. "The death trend involves the death or loss under dramatic and often tragic circumstances of individuals closely related to the patient, generally parents, siblings, and mates." Only 5 of the 25 students in this study had lost a parent or close relation. Two of these patients suggest a strong death trend. One student expressed a morbid longing for a younger sister who died seven years earlier, and another student committed suicide on the birthday of a former roommate who had committed suicide two years earlier.

DISCUSSION

Suicide is an important public health problem among college students; it is the second most common cause of death in this group, accounting for 8 to 12 per cent of all deaths. Students in college are faced with many psychological and emotional stresses: they are usually away from home for the first time and have to adjust to a new environment, make new friends, and plan their own personal lives; they must adjust from a dependent mode of existence to an independent one; adjustments and attitudes towards the opposite sex are being worked out; new ideas related to life, customs, mores, and religion are presented and often conflict with what has been taught at home; and often for the first time the student begins to realize the

importance of doing well in his academic studies because his future livelihood depends on it. Fry[7] mentioned that a student has the following adjustments to make: sexual and behavioral adjustments, family adjustments, scholastic adjustments, and social adjustments. Many students find it impossible to make these unaided. Farnsworth[6] estimated 10 per cent of all college students need professional help with their conflicts and emotional problems.

American colleges and universities have not assumed their full share of responsibility where the physical and mental health of their student population is concerned. It was estimated from a 1953 survey of 1,157 colleges that one in three has no clinical service, one in three has clinical services for minor disorders, and one in three has clinical services for both major and minor disorders.[10] When one realizes that there are approximately two and one-half million students enrolled in American colleges, the problem of preventive psychiatry and mental hygiene becomes a stimulating challenge. Abnormal behavior can be diagnosed early in a college setting—the problem is to teach students and faculty members the basic principles of mental hygiene and to provide students with adequate psychiatric counseling services.

SUMMARY

1. This study found that suicide accounted for 25, or 12 per cent, of 209 deaths among college students from 1920-1955. The death rate from suicides among college students is not significantly higher than a comparable group of the noncollege population.

2. Of the 25 suicides investigated in this study, 40 per cent committed suicide in the college community, 40 per cent at home, and 20 per cent away from both home and college. Ninety-six per cent of them selected one of four methods for self-destruction—firearms, hanging, asphyxiation by gas, or poisoning.

3. Though symptoms of emotional upset are frequently observed in a college environment, only 40 per cent of the patients who eventually took their lives were referred for psychiatric treatment. Only 6 of the 25 students in this study were brilliant in their studies; most of the students who committed suicide were average students.

4. Financial worries, sexual adjustments, scholastic difficulties, and family adjustments were significant areas of added stress in the lives of the students in this study.

5. The college community provides an excellent setting for preventive psychiatry and mental hygiene, but American colleges have not assumed their full share of responsibility for this problem.

ACKNOWLEDGMENT

The author would like to express his gratitude to Dr. Bryant M. Wedge, Acting Chief of the Department of Mental Hygiene and Psychiatry, Yale University, for his suggestions and co-operation in this study.

REFERENCES

1. Collins, S. D., Lehmann, J. L., and Trantham, K. S.: *Major causes of death in six age groups of life.* Washington, D. C., U. S. Government Printing Office (Public Health Monograph No. 30), 1955, p. 14.
2. Dennison, S. R.: *Suicides among students.* Editorial in *The London Times,* August 28, 1953.
3. Diehl, H. S. and Shepard, C. E.: *The health of college students.* Washington, D. C., American Council on Education, 1939, p. 100.
4. Dublin, L. I.: *The facts of life from birth to death.* New York, Macmillan Co., 1951, p. 262.
5. Dublin, L. I. and Bunzel, B.: *To be or not to be.* New York, Smith & Haas, 1933.
6. Farnsworth, D. L.: Health on the campus. Bull. nat. Tuberc. Ass., 1956, *42,* 71-72.
7. Fry, C. C.: *Mental health in college.* New York, Commonwealth Fund, 1942.
8. Gordon, J. E. *et al.*: *Epidemiology of mental disorder.* New York, Millbank Memorial Fund, 1950.
9. Henn, T. R.: *Suicides among students.* Editorial in *The London Times,* August 28, 1953.
10. Moore, N. S. and Summerskill, J.: *Health services in American colleges and universities.* Ithaca, N. Y., Cornell University, 1954.
11. Moss, L. M. and Hamilton, D. M.: The psychotherapy of the suicide patient. Amer. J. Psychiat., 1956, *112,* 814-820.
12. Parrish, H. M.: Causes of death among college students. Pub. Hlth. Rep. Wash., 1956, *71,* 1081-1085.
13. Statist. Bull. Metropolitan Life Insur. Co.: Why do people kill themselves?, 1945, *26,* 9-10.

HARRIS BUSCH*

Department of Pharmacology, University of Illinois College of Medicine, Chicago, Illinois

BIOCHEMICAL ASPECTS OF PANCREATITIS**

In its acute forms, pancreatitis is a severe and frequently fatal disease, characterized biochemically by intrapancreatic activation of very powerful digestive enzymes.[55] The activation of these enzymes results in hydrolytic cleavage of cellular structures followed by generalized pancreatic necrosis, peritonitis, and vasomotor collapse.[68, 72] In its chronic forms, pancreatitis is a disabling disease associated with progressive digestive insufficiency due to decreasing concentrations of pancreatic enzymes in the intestinal contents.[20] Accordingly, malnutrition, anemia, and avitaminosis accompany the recurrent epigastric pain which results from localized pancreatic inflammation.

INTRACELLULAR METABOLISM OF THE PANCREAS

The enzymes and external secretion of the pancreas are produced by the acinar cells.[65] For an adequate understanding of pancreatitis, a great deal must be learned about the specific metabolism of these cells, inasmuch as at present there are relatively few established facts. The *oxidative metabolism* of the pancreas is apparently very similar to that of other tissues as indicated by (i) a QO_2 for pancreas slices of 6-7;[15, 20, 122] (ii) accumulation of citrate in the pancreas of the fluoroacetate-treated animal; and (iii) accumulation of isotope of labeled acetate and labeled pyruvate in the glutamate pool of the pancreas.[111] Evidence for very active *protein synthesis* has been obtained from studies in which isotopically labeled amino acids have been injected into animals *in vivo*. In the rat, labeling of protein with isotopically labeled lysine or glycine was greater in the pancreas than in any other tissue studied;[19] in the dog, only the duodenal mucosa was as active in labeling of protein.[58] The quantity of protein produced by the human pancreas has been calculated as 10-20 grams, i.e., one to two liters of exocrine secretion daily with a 1-2 per cent concentration of enzymes.[14, 20, 21] In some animals 20 per cent of the dry weight of the gland consists of enzymes with

---

* Associate Professor of Pharmacology.

** Some of the original studies reported were supported by grants from the Jane Coffin Childs Fund.

Received for publication December 12, 1956.

trypsin, amylase, lipase, and ribonuclease comprising 10, 5, 2½, and ½ per cent respectively of the dry weight.[88] Attempts to study synthesis of pancreatic enzymes *in vitro* have only gone as far as demonstration of enzyme synthesis by pancreatic slices. Hokin initially injected animals with cholinergic compounds to deplete the pancreas of enzymes;[75, 76] he then incubated pancreas slices in media supplemented with amino acids, and found that the enzymatic concentration increased in the medium after the slice had been stimulated with carbamyl choline.

THE EXOCRINE SECRETION OF THE PANCREAS

As indicated in Table 1, the exocrine secretion of the pancreas contains a variety of hydrolytic enzymes, including the *peptidases,* trypsin, chymotrypsin, and carboxypeptidase, the *glycosidases,* amylase and maltase, the *esterases,* cholesterol esterase and lipase, and the *nucleases,* ribonucleases and deoxyribonuclease.[80]

Peptidases. Trypsin and chymotrypsin are normally present in the pancreas as the inactive precursors, i.e., trypsinogen and chymotrypsinogen. Activation of both enzymes results from hydrolysis of a "protective" peptide from the main enzyme molecule. Trypsinogen is a protein which contains the peptide chain, valyl -aspartyl-aspartyl-aspartyl-aspartyl-lysyl-isoleucine; in its activation to trypsin by trypsin, hydrolysis of the lysyl-isoleucyl bond results in the liberation of the active trypsin and the free hexapeptide, valyl-aspartyl-aspartyl-aspartyl-aspartyl-lysine.[85] In the duodenum and jejunum, trypsinogen is converted to active trypsin by the enzyme enterokinase.[85, 87, 94, 107] The mechanism of the activation is not established, but is not simply a release from the inhibition of pancreatic trypsin inhibitor.[94] Since trypsin itself can activate trypsinogen as noted above, the process is autocatalytic once started; calcium ion is an activator for the reaction.[20, 85] Trypsin is an endopeptidase attacking proteins either at the center or ends of the molecule at peptide bonds of the basic amino acids, arginine, and lysine on the amide linkage joining the carboxyl of these amino acids to the adjacent amino acid (Table 1).[9] The proteolytic activity of trypsin has been applied therapeutically for enzymatic debridement of ulcerating or other chronic infections.[90]

Chymotrypsinogen is a precursor of the proteinases grouped as chymotrypsin (Table 1). In the course of its activation by trypsin, the "protective" peptide side chain is split off gradually resulting in a series of active chymotrypsins, including alpha, beta, and gamma chymotrypsins,[86] pi chymotrypsin,[21] and delta chymotrypsin.[118] Alpha chymotrypsinogen is a crystalline compound containing a peptide chain, leucyl-seryl-arginyl-isoleucyl-valine. In the pi chymotrypsin, the terminal isoleucyl-valine dipeptide has been

split off the parent protein, while the delta form is the result of the loss of the seryl-arginine dipeptide.[44] The chymotrypsins differ from trypsin in their ability to clot milk; their substrate specificity is also different from that of trypsin, since they require either an aromatic ring, such as that found in tryosine,[9] phenylalanine, and tryptophan or methionine[118] adjacent to the peptide bond which they attack.

Carboxypeptidase. In addition to the endopeptidases, trypsin and chymotrypsin, the pancreas also secretes carboxypeptidase, an exopeptidase which attacks only terminal peptide bonds. This enzyme attacks the terminal peptide bond of a chain of amino acids at the carboxyl end of a polypeptide.[74]

Trypsin inhibitors. The first of the trypsin inhibitors was isolated and crystallized from pancreas by Kunitz and Northrop and was shown to combine with trypsin stoichiometrically to yield a third protein possessing no proteolytic activity.[68] Inhibitors of trypsin have been isolated from a variety of sources, including soybeans, lima beans, and intestinal parasites such as Ascaris.[80, 83, 90] These compounds are polypeptides or proteins with molecular weights ranging from 8,000-40,000 and they are thought to combine with trypsin in a 1 : 1 ratio. The tenacity of their binding with trypsin is evident from the dissociation constant of the trypsin-inhibitor (pancreatic) complexes which have been estimated as 2×10^{-10} M.[63, 64, 65, 67] The inhibition was originally thought to be noncompetitive, but Green has shown that the ethyl ester of the substrate, benzoyl-L-arganineamide, protects the enzyme against the pancreatic inhibitor. Green also found that DFP (diisopropylfluorophosphate), which itself inhibits tryptic activity, protects the enzyme against combination with the pancreatic inhibitor and suggested that the inhibitor combines with the enzyme at the "active site."[63, 90] Chymotrypsin is also inhibited by DFP.

Tests of tryptic activity. Two clinical methods are useful for testing for intestinal activity of this enzyme group. In the first, hydrolysis of gelatin is measured either by a viscosimetric procedure which estimates liquefaction of gelatin[6] or by the clearing of a spot on the x-ray film by a known dilution of a fecal suspension.[116] In each instance, a negative test is of little significance, since trypsin itself is hydrolyzed in the passage of the intestinal contents through the gut. The methods are most applicable in pediatric practice, since the stools of infants are generally soft and moist; in the adult, desiccation and hardening of the stool is associated with destruction of the enzyme.[74] A second method for the determination of proteolytic activity is the study of the stool for meat fibers (creatorrhea).[74] This test is of some use in testing for pancreatic insufficiency.

TABLE 1. EXOCRINE PANCREATIC SECRETIONS

Compound	*Activators*	*Inhibitors*	*Control mechanism*	*Bonds attacked*	*Tests for activity*
I. *Enzymes*					
A. Proteinases					
1. Trypsinogen	Enterokinase trypsin, Ca++	Pancreatic, blood, soybean, DFP	I. Vagus, cholinergic drugs	Peptides of arginine, lysine, on amide link	1. Microscopic stool for fibers 2. Gelatin film
2. Chymotrypsinogen	Trypsin	DFP	II. Pancreozymin	Clots milk; attacks peptides of amino acids with aromatic ring	Gelatin film
3. Carboxypeptidase	Mg++, SH	Iodoacetic acid, CN—, PO_4		Terminal peptide bond on carboxyl end of polypeptide	Gelatin film
B. Lipase	Ca++, Bile salts			ester bonds of triglycerides	Olive oil hydrolysis, stool fat, stool fatty acids
C. Amylase	Cl—			α-1,4 glucosides	Starch hydrolysis
D. Nucleases					
1. Ribonuclease					
2. Deoxyribonuclease					
II. *Inorganic Compounds*					
1. Bicarbonate		Diamox	Secretin		Duodenal aspirate after secretin for: (1) HCO_3 (2) volume
2. Water		Diamox			

Glycosidases. The most important of the pancreatic glycosidases is the enzyme pancreatic amylase (Table 1).[108] This enzyme has a molecular weight of 45,000 and has the characteristics of an alpha amylase, i.e., it attacks the internal bonds of the polysaccharide and does not release single glucose units.[108, 122] The enzyme specifically attacks the α-1, 4-glucosidic bond and exhibits no significant activity on structures with the β-glycosidic linkage. Pancreatic amylase hydrolyzes starch to maltose and trisaccharides and is activated fourfold by the addition of chloride ion. As far as can be determined, pancreatic amylase is the same enzyme as salivary amylase. The products of the hydrolytic activity of pancreatic amylase are hydrolyzed to glucose by maltase.

Tests for amylase activity. The clinical methods applied to the determination of amylase activity at present are "saccharogenic" or "amyloclastic."[102, 110, 122] The method of Somogyi determines the quantity of glucose or reducing sugar liberated from an excess of buffered starch in 30 minutes at 38° C. The results, expressed in terms of mg. of glucose liberated per 100 cc. serum, are 70-200 Units for normal serum. In the "amyloclastic" method of Wohlgemuth, a series of dilutions of the serum are incubated with a limiting quantity of starch substrate for 30 minutes at 38° C. At the end of the incubation the degree of hydrolysis of the starch in each tube is determined by addition of a drop of iodine; the highest dilution of serum which results in a colorless solution on the addition of iodine is the end-point; the inverse of the dilution is reported as the number of units in the serum. Normally, 8-16 Wohlgemuth units of amylase activity are present per 100 cc. of serum. Both methods are quite reliable as indexes of amylase activity, but there seems little to choose between the two methods, inasmuch as the Somogyi procedure has the advantage of sensitivity, while the Wohlgemuth procedure has the virtue of simplicity.

Lipase. Pancreatic lipase is an esterase which is apparently the only lipase secreted in significant quantity or activity in the gastrointestinal tract. In general, it is more active toward triglycerides containing long chain, unsaturated fatty acids. The end-products of its activity are a mixture of diglycerides, monoglycerides, fatty acids, and glycerol. The optimum pH for the enzyme is 7-8 and the activity is enhanced by the addition of bile salts, calcium ion, and various peptides to the medium. Following the report of Elman on the usefulness of amylase studies for diagnosis of pancreatic inflammation, Cherry and Crandall reported an increase in plasma lipase activity in pancreatic obstruction.[22] The technique used for determination of the activity of the enzyme requires a 24-hour incubation of 5 cc. of a buffered suspension of olive oil with 1 cc. of serum at 40° followed by titration

of the liberated fatty acids. Unfortunately, the long incubation period has precluded the use of this technique as a routine procedure[74] and more soluble substrates are being sought in an effort to shorten the required incubation time.[68, 117] In chronic pancreatic insufficiency, the lack of pancreatic lipase results in steatorrhea and the excretion of foul, greasy stools. The fat may be seen microscopically by staining the stool with Sudan IV or it may be determined gravimetrically;[74] an increase in the percentage of lipid from the normal value of 10-20 to 40-50 per cent of the total dry weight of the stool is very suggestive of pancreatic disease.[4, 27, 30, 80]

In addition to the enzymes discussed above, the pancreatic secretion contains enzymes which have been less extensively studied, both from a clinical or biochemical point of view; these include cholesterol esterase, ribonuclease, and deoxyribonuclease. The endocrine secretion of the pancreas contains two other polypeptides, insulin and glucagon, but these play no apparent rôle in pancreatitis.

The *inorganic compounds* secreted by the pancreas are chiefly water and the ions normally found in the serum. Under the influence of secretin, the secretion of water increases markedly, and the bicarbonate ion concentration rises to the level of the chloride ion, i.e., from 6-30 mEq./liter to 120-130 mEq./liter, while the chloride ion concentration drops from 120 mEq./liter to 28 mEq./liter.[1, 2, 99] In the secretin test for the study of pancreatic secretion, the average volume of secretion is 250 cc., generally containing 32 mEq. of base as alkali reserve. In the test meal for gastric acidity, an average of 180 cc. of 0.1 N base are required to neutralize the gastric secretion, i.e., 18 mEq. of acid are formed. The approximate equivalence of these values has been the source of speculation on the rôle of the pancreas in maintaining the normal duodenal pH and preventing peptic ulcer.[59, 120] It is thought that the circulating blood is the source of the bicarbonate of the pancreatic secretion, inasmuch as $C^{14}O_2$ injected into secretin-treated animals appears promptly in the pancreatic secretion. Although the mechanism of transfer of the bicarbonate from the blood to the pancreatic secretion is unknown, it is suspected that carbonic anhydrase may play an important rôle, inasmuch as Diamox ® inhibits both the increase in bicarbonate as well as the increase in total volume.[18]

PHYSIOLOGICAL CONTROLS OF THE EXOCRINE SECRETION

Secretin. Pavlov and his co-workers found that the flow of pancreatic secretion was initiated by the entry of gastric contents into the duodenum.[107] They also found that the pancreatic secretion varied with the diet, so that animals fed bread exhibited increased amylolytic activity, while a meat diet

evoked the maximum tryptic activity. While this challenging observation has not been explained, it has been verified by other workers since that time.[99] Bayliss and Starling found that the increase in secretion evoked by acid was mediated by secretin,[8] a polypeptide hormone formed by the duodenal mucosa and released into the blood stream in the presence of acid. Secretin has been crystallized and has been found to have a molecular weight of 5,000.[8, 70] Its amino acid composition has been studied by Agren and Willander who found that it contained much lysine and arginine, but no cystine, tyrosine, or tryptophan.[3]

Secretin test for pancreatic function. The chief effects of secretin are the increases in volume and bicarbonate content of the pancreatic secretion and these effects are the parameters used in the secretin test for pancreatic function introduced clinically by Agren and Lagerlof.[1, 2, 97] Following the intravenous injection of 0.75 mg/kg or 1 U/kg of the hormone, collection of the pancreatic secretion is carried out via a biluminal tube for an 80-minute period, in 1, 2, or 4 equal parts;[1, 2, 7, 99] the outer lumen of the tube separates gastric secretion from the duodenal contents which are collected from the center lumen. The response should include the secretion of a volume of 125-250 ml., with a bicarbonate concentration of 90-130 mEq./l., 90-1,200 U. of amylase, 20-120 U. of trypsin, and 7,000-14,000 U. of lipase.[97, 48, 91]

Vagal stimulation. The physiological controls of the secretion of pancreatic enzymes have been less extensively studied. Mellanby and others noted that stimulation of the vagus nerves or administration of cholinergic compounds results in the secretion of a small quantity of fluid rich in enzymes;[95, 97] this secretion was found to be accompanied by discharge of zymogen granules of the pancreatic acinar cells. Similar effects have been obtained by the hormone pancreozymin which has been isolated from the small intestine by Harper and Raper.[66, 71] The relative rôle of the nerve impulses and hormones is not yet evaluated.

PATHOGENESIS

The acute inflammatory diseases of the pancreas are *acute pancreatic necrosis* (acute hemorrhagic pancreatitis) and *acute interstitial pancreatitis* (acute pancreatic edema). The significant development in pancreatitis is the release of free trypsin in the pancreatic acini or cells. The evidence for the key rôle of trypsin was obtained in experiments of Polya and Rich and Duff.[109, 110] In 1905 Polya reported that the injection of as little as 1 ml. of a solution of trypsin into the pancreatic duct of dogs resulted in fatal acute hemorrhagic pancreatitis, while injection of greater volumes of inactivated

preparations did not result in such lesions. Rich and Duff pointed out that trypsin, unlike pepsin, produced severe local hemorrhage,[93, 121, 84] edema, and necrosis.

Mechanism of release of trypsin. The basic question in the etiology of pancreatitis is what is the mechanism of activation and/or release of the trypsin? Kunitz has shown that crystalline trypsinogen continuously undergoes spontaneous breakdown to yield trypsin and this process is catalyzed by ammonium sulfate or magnesium sulfate.[85] If this process were not rapidly and effectively suppressed in the pancreatic cells, acute pancreatitis would be a far more common clinical event than it is at present. A relatively small number of trypsin molecules would "autocatalyze" activation of a progressively increasing number of molecules to trypsin and chymotrypsin.[86] It can be presumed that the large quantity of very effective pancreatic trypsin inhibitor suppresses this process in its inception. Evidence for this concept has come from studies of Kalser and Grossman who produced pancreatitis by daily administration of ethionine, an analogue inhibitor of methionine metabolism.[80] They found an increase in the concentration of active trypsin in the pancreatic secretion and in the pancreas. Significantly, they found a depression in the ratio of the concentration of trypsin inhibitor to trypsin, both in the pancreatic secretion and in the pancreatic tissue.

These studies do not provide insight into the mechanism of reduction of trypsin inhibitor in human pancreatitis. However, a number of other theories for pancreatitis relate indirectly to this question. The older theories of pathogenesis of pancreatitis include: (i) the "obstruction-hypertension theory" which suggests that the pressure is elevated in the pancreatic ducts and function is suppressed by the ensuing increased cellular pressure;[21, 105, 114, 160] (ii) the "common channel theory" which suggests that there may exist a common channel between the bile ducts and the pancreatic ducts with bile acting as a hypothetical activating agent for trypsin;[53, 98] and (iii) the "oversecretion" theory which is supposed to account for the relationship of pancreatitis to alcoholism.[35, 38, 42, 43, 60] Each of these possibilities may account for a temporary cessation of formation of trypsin inhibitor. If this inhibitor is normally the rate-limiting factor for activation of trypsinogen, its absence would result in a sudden increase in active trypsin in the gland, and thereby set off the whole hydrolytic process.

The chronic inflammation of the pancreas, *chronic relapsing pancreatitis,* is divided into two stages of *recurrent pancreatitis and pancreatic insufficiency.* Recurrent pancreatitis is considered to be a more localized acute interstitial pancreatitis, while the end-stage of pancreatic insufficiency results from fibrosis of the pancreas (Table 2).

TABLE 2.

Pathogenesis	*Clinical signs*	*Laboratory findings*	*Therapy*
	ACUTE PANCREATIC INFLAMMATION		
I. *Intrapancreatic* decreased Trypsin inhib.			
1. tryptic hydrolysis of gland, edema	violent epigastric pain		1. Meperidine, opiates 2. Nerve blocks 3. Atropine-like drugs
2. peptone formation, hemorrhage	shock, hyperthermia	depressed serum Ca	Blood, plasma, fluid and electrolyte
3. lipolysis, calcium soaps			20% calcium lactate I.V.
II. *Extrapancreatic*			
1. Intraperitoneal enzymes			
a. omental fat necrosis	abdominal pain, nausea, vomiting, distention, rigidity	increased levels of pancreatic enzymes in peritoneal fluid	Pancreatic rest: nothing orally, nasogastric suction, phenobarb., atropine-like drugs
b. peritoneal irritation	ileus	x-ray: ileus	
2. Plasma enzymes	shock (peptones?)	amylase: 500-3000 U. lipase: 5-15 U. increased urinary amylase, lipase	Trypsin inhibitor?
3. Bacterial invasion, cholangiitis, abscess	jaundice, sepsis		For secondary infection: penicillin, aureomycin, terramycin
	CHRONIC PANCREATIC INSUFFICIENCY		
I. Insufficient lipase	malnutrition, steatorrhea	increases stool fat decreased fatty acids	high caloric diet, pancreatic enzymes (pancreatin, N.F.)
II. Insufficient tryptic activity	anemia	creatorrhea, azotorrhea	raw pancreas?
III. Fibrosis, calcification	palpable pancreas, diabetes	glycosuria, x-ray calcification	NPH insulin

CHEMICAL PATHOLOGY OF PANCREATITIS

Acute pancreatitis. The destruction of pancreatic tissue by proteolytic enzymes not only produces hemorrhage and necrosis,[38, 48] but also liberates an active pool of other enzymes, including pancreatic lipase. The release of lipase is accompanied by hydrolysis of fat of the pancreatic tissue, the omentum and the mesentery. The freed fatty acids rapidly form insoluble calcium soaps by combination with calcium ion of the circulating blood.[116] The formation of calcium soaps depletes the calcium ion in the plasma as was shown by Edmondson and Fields who found 100-1730 mg. of calcium in the pancreas of patients with pancreatitis.[46] Inasmuch as there is approximately 10 mg. calcium per 100 cc. plasma, the total in the blood of a 70 kg. man would be 350 mg. in 3.5 liters of plasma. The extracellular fluid contains approximately 5 mg./100 cc. or 575 mg. in 11.5 liters. Accordingly, the combined total for plasma and extracellular fluid is 925 mg. The large reservoir of calcium in the bones could easily replace the losses from the plasma and extracellular fluid in time, but the rate of replacement generally does not equal the rate of depletion. Edmondson and Berne found that the plasma level of calcium dropped to less than 9 mg./100 cc. in 72 per cent of cases studied and in a number of cases, levels of less than 7 mg./100 cc. were noted; tetany has been reported.[46, 47]

Serum levels of enzymes. The studies of Elman and coworkers, first reported in 1929, established the importance of the determination of the concentration of amylase in the blood for the diagnosis of acute pancreatitis.[50, 53] It has been pointed out that normally all exocrine enzymes of the pancreas are found in small amounts in the blood;[79] however, pancreatitis alters the so-called "exocrine-endocrine partition" in the direction of an increased blood amylase, lipase, and possibly trypsin concentration. Elman *et al.* measured the rate of decrease in the viscosity of a starch solution as an indication of the concentration of amylase; they found an increase of 2- to 20-fold in activity of amylase in the serum of patients with acute pancreatitis. In terms of modern analytical values, the serum amylase increases from an average value of 150 Somogyi Units/100 ml. plasma to more than 500 U/100 ml. of plasma, and maximum values of 8,000 U/100 cc. plasma have been reported.[15, 112, 134] In acute pancreatitis, these values remain elevated for 48-72 hours and then decrease to a normal level.[53] The pancreatic amylase is excreted by the kidney and within two hours after the serum level has risen, the level in the urine increases; a confirmatory test for pancreatitis has been developed utilizing the urine amylase level.[54, 73, 83] A marked rise in lipase of the blood is also noted and the level of this enzyme remains elevated for a considerably longer time than does the amylase level.[80, 84, 74, 91]

This test for pancreatic inflammation has been reported as 95-97 per cent positive. Thus far, a significant increase in trypsin level in the blood has not been found. It may be that the trypsin inhibitor in the blood complicates accurate estimation of this enzyme in the blood.[77, 78]

Chronic pancreatitis. In chronic pancreatitis, the progressive destruction of acinar tissue results in a decreased secretion of pancreatic enzymes. A similar pancreatic insufficiency is found in cystic fibrosis of the pancreas.[4] Steatorrhea, azotorrhea, and creatorrhea result in the formation of a bulky, greasy, foul stool, weight loss, and polyphagia.[28, 108] Amylorrhea is not found, indicating the presence of adequate digestive enzymes for polysaccharides in the intestinal secretions. The presence or absence of creatorrhea, simply determined by observation of undigested meat fibers in the stool, may also be of significance in differential diagnosis of biliary deficiency and sprue, since creatorrhea does not accompany these diseases. The percentage as "split fat," or fatty acids, is low in pancreatic disease and this may be a useful point in differentiating sprue or biliary deficiency.[29, 42, 45, 101] A result of steatorrhea is deficient absorption of fat soluble vitamins. After administration of Vitamin A to control subjects, the level in the plasma increased to 300 micrograms/100 ml. plasma from a control level of 50-100 micrograms/100 ml.[61] In contrast, a failure of increase in the plasma level of Vitamin A was noted in patients with cystic fibrosis of the pancreas or celiac disease. Administration of pancreatin resulted in a marked increase in the level of Vitamin A in the plasma of patients with cystic fibrosis of the pancreas.

THERAPY

Acute pancreatitis. Since there are no readily available specific inhibitors for pancreatic enzymes,[88, 104, 110, 115] the therapy for acute pancreatitis is primarily supportive.[23, 27, 28, 92, 108] This supportive therapy can be summarized in four basic principles: (i) relief of pain, (ii) inhibition of pancreatic secretion, (iii) restoration of nutritional, fluid, and electrolyte balance, and (iv) prevention of infection (Table 2).[10, 21, 42, 54, 100] Surgical procedures are limited to complications of the disease.[21, 51, 106] For relief of pain,[88] meperidine (Demerol) or opiates are administered parenterally, and local anesthesia has been employed by means of epidural, paravertebral, and splanchnic nerve blocks. To suppress liberation of secretin, oral feeding is prohibited and nasogastric suction is instituted. Vagal stimulation of the pancreas and the sphincter of Oddi is depressed by administration of atropine and atropine-like drugs.[6, 42, 96] Fluids and electrolytes are administered in quantities sufficient to compensate both for the daily secretions and the gastric aspirations. Blood calcium levels are maintained at normal values by administration of 10 per cent

calcium gluconate. Blood or plasma are used to treat attendant shock.[21, 66] For therapy of secondary infections, antibiotics have been used either singly or in combination. Supportive therapy with cortisone and ACTH has also been studied.[10, 49] In the recovery period, antacids are administered and stimulants of gastric secretion, such as coffee and alcohol, are omitted from the diet.

Chronic pancreatitis. In the various forms of pancreatic insufficiency, maintenance of nutrition is an important part of medical therapy. Replacement therapy with pancreatic enzymes has been used to combat the severe weight loss which accompanies pancreatic insufficiency. Raw pancreas or enteric coated pancreatin (N.F.) is administered orally in doses of 10-20 grams daily.[24, 26] While excellent amylolytic, lipolytic, and proteolytic activity has been reported for some preparations of pancreatic enzymes, carefully controlled studies on their fate and effectiveness are uncommon. The remainder of therapy is symptomatic and designed to prevent further attacks. Surgery has a limited rôle in therapy of chronic pancreatic inflammation.[21, 39, 40, 41, 57] Atropine-like drugs and analgesics may be utilized for relief of distress. Antacids may be administered and alcohol is prohibited. The mild diabetes which occasionally occurs is readily controllable with low doses of insulin.[58]

In summarizing therapy of pancreatitis, it is apparent that marked gaps exist between biochemical knowledge of normal pancreatic function, pathogenesis, and chemical pathology of the disease on the one hand, and the availability of specific therapeutic agents on the other. As biochemical pharmacodynamics advances, it seems reasonable to hope that useful drugs will become available which will inhibit the necrotizing effects of trypsin and other released pancreatic enzymes in acute pancreatitis. A more readily approachable problem would be the development of methods for preparation of very active purified enzymes for treatment of chronic pancreatitis.

REFERENCES

1. Agren, G. and Lagerlof, H.: The pancreatic secretion in man after intravenous administration of secretin. Acta med. scand., 1936, *90*, 1.
2. Agren, G., Lagerlof, H., and Berglund, H.: Secretin test of pancreatic function in the diagnosis of pancreatic disease. Acta med. scand., 1936, *90*, 224.
3. Agren, G. and Willander, O.: Reinigung von Sekretin. Biochem. Z., 1933, *259*, 365.
4. Anderson, D. H.: Cystic fibrosis of the pancreas and its relation to celiac disease; clinical and pathologic study. Amer. J. Dis. Child., 1938, *56*, 344.
5. Anderson, D. H. and Early, M. V.: Method for assaying trypsin suitable for routine use in diagnosis of congenital pancreatic deficiency. Amer. J. Dis. Child., 1942, *63*, 891.
6. Annis, D. and Hallenbeck, G. A.: Some effects of Banthine on pancreatic secretion. Gastroenterology, 1951, *17*, 561.

7. Baumann, L.: *The diagnosis of pancreatic disease.* Philadelphia, J. P. Lippincott Co., 1949.
8. Bayliss, W. M. and Starling, E. H.: The mechanism of pancreatic secretion. J. Physiol. (Lond.), 1902, *28,* 325.
9. Bergmann, M., Fruton, J. S., and Pollik, H.: The specificity of trypsin. J. biol. Chem., 1939, *127,* 643.
10. Berk, J. E.: The management of acute pancreatitis. J. Amer. med. Ass., 1953, *152,* 1.
11. Bettelheim, F. R. and Neurath, H.: The rapid activation of chymotrypsinogen. J. biol. Chem., 1955, *212,* 241.
12. Birnbaum, D. and Hollander, F.: Inhibition of pancreatic secretion by the carbonic anhydrase inhibitor 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide, Diamox (#6063). Amer. J. Physiol., 1953, *174,* 191.
13. Bliss, W. R., Burch, B., Martin, M. M., and Zollinger, R. M.: Localization of referred pancreatic pain induced by electrical stimulation. Gastroenterology, 1950, *16,* 317.
14. Bodansky, M. and Bodansky, O.: *Biochemistry of disease.* New York, The Macmillan Co., 1952.
15. Bogoch, A., Roth, J. L. A., and Bockus, H. L.: The effects of morphine on serum amylase and lipase. Gastroenterology, 1954, *26,* 697.
16. Boyd, W.: *A textbook of pathology.* Philadelphia, Lea and Febiger, 1953.
17. Brown, R. K., Moseley, V., Pratt, T. D., and Pratt, J. H.: The early diagnosis of cancer of the pancreas based on the clinical and pathological study of 100 autopsied cases. Amer. J. med. Sc., 1952, *233,* 349.
18. Busch, H. and Baltrush, H. A.: Rates of metabolism of acetate-1-C^{14} in tissues *in vivo.* Cancer Res., 1954, *14,* 448.
19. Busch, H. and Greene, H. S. N.: Studies on the metabolism of plasma proteins in tumor-bearing rats. Yale J. Biol. Med., 1955, *27,* 339.
20. Cantarow, A. and Schepartz, B.: *Biochemistry.* Philadelphia, W. B. Saunders Co., 1954.
21. Cattell, R. B. and Warren, K. W.: *Surgery of the pancreas.* Philadelphia, W. B. Saunders, 1953.
22. Cherry, I. S. and Crandall, L. A., Jr.: The specificity of pancreatic lipase: Its appearance in blood after pancreatic injury. Amer. J. Physiol., 1932, *100,* 266.
23. Christopher, F.: *Textbook of surgery.* Philadelphia, W. B. Saunders, 1944.
24. Clay, W. A., Frank, D. W., and Logan, V. W.: Studies on the therapy of pancreatic insufficiency. Gastroenterology, 1954, *26,* 198.
25. Coffey, R. J., Barnes, E. B., Reed, P., and Brandt, F. B.: Diagnosis and treatment of acute pancreatic disease. Postgrad. Med., 1950, *8,* 191.
26. Coffey, R. J. and Madigan, H. S.: Treatment of chronic pancreatitis. Postgrad. Med., 1950, *7,* 436.
27. Cole, W. H. and Elman, R.: *Textbook of general surgery.* New York, D. Appleton Century, 1944.
28. Comfort, M. W.: Chronic relapsing pancreatitis: Its clinical course, sequelae, diagnosis and medical treatment in cases in which disease of the biliary tract did not coexist. Proc. Mayo Clin., 1947, *22,* 548.
29. Comfort, M. W., Gambill, E. E., and Baggenstoss, A. H.: Chronic relapsing pancreatitis: A study of 25 cases without associated disease of the biliary and gastrointestinal tract. Gastroenterology, 1946, *6,* 239, 376.
30. Comfort, M. W. and Osterberg, A. E.: The value of determination of the concentration of serum amylase and serum lipase in the diagnosis of disease of the pancreas. Proc. Mayo Clin., 1940, *15,* 427.
31. Crider, J. O. and Thomas, J. E.: Response of dogs pancreas to secretin during periods of induced hyperglycemia. Fed. Proc., 1952, *11,* 29.
32. Cullen, T. S.: A new sign in extra-uterine pregnancy. Amer. J. Obst., 1918, *78,* 457.
33. Daly, M. M. and Mirsky, A. E.: Formation of protein in the pancreas. J. gen. Physiol., 1952, *36,* 243.
34. Dankner, A. and Heifetz, C. J.: The interrelationship of blood and urinary diastase during acute transient pancreatitis. Gastroenterology, 1951, *18,* 207.

35. Davie, F. W. and Neurath, H.: Identification of a peptide released during autocatalytic activation of trypsinogen. J. biol. Chem., 1955, *212,* 515.
36. Demel, R.: Der derzeitige standpunkt in der behandlung der acuten pankreasenekrose. Med. Klin., 1941, *37,* 404.
37. Diamond, J. S. and Siegel, S. A.: The secretin test in the diagnosis of pancreatic disease with a report of one hundred thirty cases. Amer. J. dig. Dis., 1940, *7,* 435.
38. Dornberger, G. R., Comfort, N. M., Wollaeger, E. E., and Power, M. H.: Total fecal solids, fats and nitrogen: A study of patients with chronic relapsing pancreatitis. Gastroenterology, 1948, *11,* 691.
39. Doubilet, H. and Mulholland, H. H.: Surgical treatment of recurrent acute pancreatitis by endocholedochal sphincterotomy. Surg. Gynec. Obstet., 1948, *86,* 295.
40. Doubilet, H. and Mulholland, J. H.: Surgical treatment of pancreatitis. Surg. Clin. N. Amer., 1949, *29,* 339.
41. Doubilet, H. and Mulholland, J. H.: The results of sphincterotomy. J. Mt Sinai Hosp., 1951, *17,* 458.
42. Dreiling, D. A. and Richman, A.: Pancreatitis; a review. J. Mt. Sinai Hosp., 1954, *21,* 122, 176.
43. Dreiling, D. A., Richman, A., and Fradkin, N. F.: The role of alcohol in the etiology of pancreatitis: A study of the effect of intravenous alcohol on the external secretion of the pancreas. Gastroenterology, 1952, *20,* 636.
44. Dreyer, W. J. and Neurath, H.: The activation of chymotrypsinogen. J. Amer. chem. Soc., 1955, *77,* 814.
45. Duff, G. L.: Clinical and pathological features of carcinoma of the body and tail of the pancreas. Bull. Johns Hopk. Hosp., 1938, *65,* 69.
46. Edmondson, H. A. and Berne, C. J.: Calcium changes in acute pancreatic necrosis. Surg. Gynec. Obstet., 1944, *79,* 240.
47. Edmondson, H. A., Berne, C. J., Homann, R. E., Jr., and Wertman, M.: Calcium, potassium, magnesium, and amylase disturbances in acute pancreatitis. Amer. J. Med., 1952, *12,* 34.
48. Edmondson, H. A. and Fields, I. A.: Relation of calcium and lipids to acute pancreatic necrosis. Report of fifteen cases in one of which fat embolism occurred. Arch. int. Med., 1942, *69,* 177.
49. Ekswith, I. S., Cacace, V. A., and Sollosy, A.: Acute hemorrhagic pancreatitis: Treatment with cortisone. New Engl. J. Med., 1955, *252,* 494.
50. Elman, R.: Variations of blood amylase during acute transient disease of the pancreas. Ann. Surg., 1937, *105,* 379.
51. Elman, R.: Surgery in acute pancreatitis. Gastroenterology, 1946, *7,* 656.
52. Elman, R., Aronson, N., and Graham, E. A.: Value of blood amylase estimations in diagnosis of pancreatic disease. Arch. Surg., 1929, *19,* 943.
53. Fallis, L. S. and Szilagyi, D. E.: Observations on some metabolic changes after total pancreaticoduodenectomy. Ann. Surg., 1948, *128,* 639.
54. Farrer, J. J.: Pancreatitis, medical aspects. Med. Clin. N. Amer., 1954, *38,* 1393.
55. Fitz, R. H.: Acute pancreatitis. Med. Rec. (N. Y.), 1889, *35,* 197, 225, 253.
56. Flexner, S.: The constituent of the bile causing pancreatitis and the effect of colloids on its action. J. exp. Med., 1906, *8,* 167.
57. Fogerson, V. and Shedd, D. P.: Acute pancreatitis. Surg. Gynec. and Obstet., in press.
58. Friedberg, F., Tarver, H., and Greenberg, D. M.: The distribution pattern of sulfur-labeled methionine in the protein and free amino acid fraction of tissues after intravenous administration. J. biol. Chem., 1948, *173,* 355.
59. Friedman, M. H. F.: The significance of the discovery of secretin. Gastroenterology, 1954, *26,* 795.
60. Gambill, E. E., Comfort, M. W., and Baggenstoff, A. H.: Chronic relapsing pancreatitis: an analysis of 27 cases associated with disease of the biliary tract. Gastroenterology, 1948, *11,* 1.
61. Gibbs, G. E.: Effect of pancreatin on plasma vitamin A curves in the celiac syndrome. Pediatrics, 1950, *6,* 593.

62. Goldstein, N. P., Epstein, J. H., and Roe, J. H.: Studies of pancreatic function; a simplified method for determination of serum lipase using aqueous tributyrin as substrate with 100 normal values by this method. J. Lab. clin. Med., 1948, *33*, 1047.
63. Green, N. M.: Competition among trypsin inhibitors. J. biol. Chem., 1953, *205*, 535.
64. Green, N. M. and Work, E.: Pancreatic trypsin inhibitor. I. Preparation and properties. Biochem. J., 1953, *54*, 257.
65. Green, N. M. and Work, E.: Pancreatic trypsin inhibitor. II. Reaction with trypsin. Biochem. J., 1953, *54*, 347.
66. Greengard, H., Grossman, M. I., Woolley, J. K., and Ivy, A. C.: A confirmation of the presence of pancreozymin in the duodenal mucosa. Science, 1944, *99*, 350.
67. Grob, D.: Proteolytic enzymes. III. Further studies on protein, polypeptide and other inhibitors of serum proteinase, leucoproteinase, trypsin and papain. J. gen. Physiol., 1949, *33*, 103.
68. Grollman, A. I., Goodman, S., and Fine, A.: Localized paralytic ileus; an early roentgen sign in acute pancreatitis. Surg. Gynec. Obstet., 1950, *91*, 65.
69. Grossman, M. I., Greengard, H., and Ivy, A. C.: The effect of dietary composition on pancreatic enzymes. Amer. J. Physiol., 1943, *138*, 676.
70. Hammarsten, E., Agren, G., Hammarsten, H., and Willander, O.: Versuche zur reinigung von sekretin. Biochem. Z., 1933, *264*, 275.
71. Harper, A. A. and Raper, H. S.: Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine. J. Physiol. (Lond.), 1943, *102*, 115.
72. Harrison, T. H.: *A textbook of medicine*. New York, The Blakiston Co., 1950, p. 1473.
73. Heifetz, C. J., Probstein, J. G., and Gray, S. H.: Clinical studies on blood diastase. II. Significance of increased blood diastase. Arch. int. Med., 1941, *67*, 819.
74. Hoffman, W. S.: *The biochemistry of clinical medicine*. Chicago, Yearbook Publishers, Inc., 1954.
75. Hokin, L. E.: Synthesis and secretion of amylase by pigeon pancreas *in vitro*. Biochem. J., 1951, *48*, 320.
76. Hokin, M. R. and Hokin, L. E.: Enzyme secretion and the incorporation of P^{32} into phospholipids of pancreas slices. J. biol. Chem., 1953, *203*, 967.
77. Innerfield, I., Angrist, A., and Benjamin, J. W.: Plasma antithrombin titers in disturbances of the pancreas. Gastroenterology, 1951, *19*, 843.
78. Innerfield, I., Angrist, A., and Benjamin, J. W.: Antithrombin titer in acute pancreatitis. Amer. J. Med., 1952, *12*, 24.
79. Janowitz, H. D. and Hollander, F.: The exocrine-endocrine partition of enzymes in the digestive tract. Gastroenterology, 1951, *17*, 591.
80. Kalser, M. L. and Grossman, M. I.: Pancreatic secretion in dogs with ethionine-induced pancreatitis. Gastroenterology, 1954, *26*, 189.
81. Kazal, L. A., Spicer, D. S., and Brahinsky, R. A.: Isolation of a crystalline trypsin-inhibitor anticoagulant protein from pancreas. J. Amer. chem. Soc., 1948, *70*, 3034.
82. Keith, L. M., Zollinger, R. M., and McCleery, R. S.: Peritoneal fluid amylase determination as an aid in diagnosis of acute pancreatitis. Arch. Surg., 1950, *61*, 930.
83. Kenwell, H. N. and Wels, P. B.: Acute hemorrhagic pancreatitis. Report of eleven consecutive cases treated with human serum albumin. Surg. Gynec. Obstet., 1953, *96*, 169.
84. Kirby, C. K., Howard, J. M., and Rhoads, J. H.: Death due to delayed hemorrhage in acute pancreatitis. Surg. Gynec. Obstet., 1955, *100*, 458.
85. Kunitz, M.: Formation of trypsin from trypsinogen by an enzyme produced by a mold of the genus penicillium. J. gen. Physiol., 1938, *21*, 601.
86. Kunitz, M.: Formation of new crystalline enzymes from chymotrypsin. Isolation of beta and gamma chymotrypsin. J. gen. Physiol., 1938, *22*, 207.

87. Kunitz, M.: Purification and concentration of enterokinase. J. gen. Physiol., 1938, *22,* 447.
88. Kunitz, M. and Northrop, J. H.: Isolation from beef pancreas of crystalline-trypsinogen, trypsin, a trypsin inhibitor and a trypsin inhibitor compound. J. gen. Physiol., 1936, *19,* 991.
89. Lagerlof, H. O.: Normal esterases and pancreatic lipase in the blood: A study with new chemical and clinical methods (A second secretin test). Acta med. scand., 1945, *120,* 407.
90. Laskowski, M. and Laskowski, M., Jr.: Naturally occurring trypsin inhibitors. Advanc. Protein Chem., 1955, *9,* 203.
91. Lopusniak, M. S. and Bockus, H. L.: A study of pancreatic serum enzymes following secretin injection in pancreatic affections. Gastroenterology, 1950, *16,* 294.
92. Machella, T. E.: Acute and chronic pancreatitis. Veterans Adm. techn. Bull., 1953, *10,* 87.
93. Mann, F. C. and Giordano, A. S.: The bile factor in pancreatitis. Arch. Surg., 1923, *6,* 1.
94. Mars, P. H., Peanasaky, R. J., and Laskowski, M.: Concerning the mechanism of action of enterokinase. Proc. Soc. exp. Biol. (N. Y.), 1953, *82,* 384.
95. Maximow, A. A. and Bloom, W.: *A textbook of histology.* Philadelphia, W. B. Saunders Co., 1953, p. 414.
96. McDonough, F. E. and O'Neill, P. B.: The therapeutic value of banthine in gastrointestinal disorders. Gastroenterology, 1951, *19,* 265.
97. Mellanby, J.: The mechanism of pancreatic digestion. The function of secretin. J. Physiol. (Lond.), 1925, *60,* 85.
98. Meng, H. C.: Preparation, utilization and importance of neutral fat emulsions in intravenous alimentation. In *A symposium on fat metabolism.* V. A. Najjar, Ed., Baltimore, Johns Hopkins Press, 1954.
99. Morani, A. D.: Trypsin, its use in general practice. J. Amer. med. Wom. Ass., 1954, *9,* 313.
100. Morse, L. J. and Achs, S.: Acute pancreatitis, a clinical evaluation and review of 154 cases. Ann. Surg., 1949, *130,* 1044.
101. Muether, R. O. and Knight, W. A., Jr.: Chronic recurrent pancreatitis. I. Clinical and laboratory aspects. Gastroenterology, 1949, *12,* 24.
102. Myers, V. C., Free, A. H., and Rosinski, F. E.: Studies on animal diastases. VI. Determination of diastase (amylase) in blood. J. biol. Chem., 1944, *154,* 39.
103. Myrback, K. and Neumiller, G.: Amylases and the hydrolysis of starch and glycogen. In *The enzymes.* J. G. Sumner and K. Myrback, Eds. New York, Academic Press, Inc., 1951.
104. Noskin, E. A., Popper, H. L., and Necheles, H.: Attempts to prevent pancreatic fat necrosis. Rev. Gastroent., 1952, *19,* 731.
105. Opie, E. L.: The relationship of cholelithiasis to diseases of the pancreas and to fat necrosis. Amer. J. med. Sci., 1901, *121,* 27.
106. Paxton, J. R. and Payne, J. H.: Acute pancreatitis; a statistical review of 307 established cases of acute pancreatitis. Surg. Gynec. Obstet., 1948, *86,* 69.
107. Pavlov, I. P.: *The work of the digestive glands.* London, C. Griffin and Co., Ltd., 1910.
108. Peterson, L. W. and Cole, W. H.: Chronic sclerosing pancreatitis causing stenosis of the common bile duct. Arch. Surg., 1945, *51,* 15.
109. Polya, E.: Die workung des trypsins auf das lebende pankreas. Pflügers Archiv. ges. physiol., 1908, *121,* 483.
110. Popper, H. L. and Necheles, H.: Prevention of pancreatic fat necrosis. Surg. Gynec. Obstet., 1953, *96,* 299.
111. Potter, V. R. and Busch, H.: Citric acid content of normal and tumor tissues *in vivo* following injection of fluoroacetate. Cancer Res., 1950, *10,* 353.
112. Raffensperger, E. C.: Elevated serum pancreatic enzyme values with primary intrinsic pancreatic disease. Ann. int. Med., 1951, *35,* 342.

113. Rich, A. R. and Duff, G. L.: Experimental and pathological studies on the pathogenesis of acute hemorrhagic pancreatitis. Bull. Johns Hopk. Hosp., 1936, *58*, 212.
114. Roberts, H. J., Baggenstoss, A. H., and Comfort, M. W.: Acute pancreatic necrosis. A clinicopathologic study. Amer. J. clin. Path., 1950, *20*, 742.
115. Rush, B. and Cliffton, E. E.: The role of trypsin in the pathogenesis of acute hemorrhagic pancreatitis and the effect of an antitryptic agent in treatment. Surgery, 1952, *31*, 349.
116. Schwachman, H., Patterson, P. R., and Laguna, J.: Studies in pancreatic fibrosis. A simple diagnostic gelatin film test for stool trypsin. Pediatrics, 1949, *4*, 222.
117. Seligman, A. M. and Machlas, M. N.: The colorimetric determination of lipase and esterase in human serum. J. clin. Invest., 1950, *29*, 31.
118. Smith, E. L.: Proteolytic enzymes. In: *The enzymes*. J. G. Sumner and K. Myrback, Eds. New York, Academic Press, Inc., 1951.
119. Somogyi, M.: Micromethods for the estimation of diastase. J. biol. Chem., 1938, *125*, 399.
120. Thomas, J. E.: *External secretion of the pancreas*. Springfield, C. C Thomas, 1950.
121. Turner, G. G.: Local discoloration of the abdominal wall as a sign of acute pancreatitis. Brit. J. Surg., 1919, *7*, 394.
122. White, A., Handler, P., Smith, E. L., and Stetten, D., Jr.: *Principles of biochemistry*. New York, McGraw-Hill Book Co., Inc., 1954.
123. Wohlgemuth, J.: Uber eine neue methode zur quantitativen bestimmung des diastatischen ferments. Biochem. Z., 1908, *9*, 1.
124. Zollinger, R. M., Keith, L. M., Jr., and Ellison, E. H.: Pancreatitis. New Engl. J. Med., 1954, *251*, 497.

PHILIP J. GRIFFIN*
SIDNEY V. RIEDER**

Department of Biochemistry, Yale University School of Medicine

A STUDY ON THE GROWTH REQUIREMENTS OF *NEISSERIA GONORRHOEAE* AND ITS CLINICAL APPLICATION†

Routine methods for the isolation of *Neisseria gonorrhoeae* employ various complex media in conjunction with devices designed to supply an atmosphere of approximately 10 per cent carbon dioxide. Recent investigations[6,7] of the CO_2 requirement of *N. gonorrhoeae* have shown that various strains of this microorganism can be grown on a Casamino-salts-glucose agar medium in the absence of CO_2, provided an extract of yeast is added. The apparent effect of yeast extract in eliminating the requirement for CO_2 could be duplicated by a combination of hypoxanthine, uracil, and oxaloacetate; however, growth in air with this combination proved inferior to the growth with yeast extract alone.

An investigation of the nature of the additional factors in yeast extract was undertaken. This report presents evidence of the necessity for tryptophan and biotin in the metabolism of *N. gonorrhoeae* and the role of oxaloacetic acid as a source of CO_2. The recognition of the importance of CO_2 and the stimulatory effect of yeast extract has made possible the development of a simplified medium and a new technique for the isolation of *N. gonorrhoeae* from clinical specimens.

MATERIALS AND METHODS

Stock cultures grown on Mueller-Hinton medium[18] were maintained in an atmosphere of CO_2 (candle-jar method). The inoculum consisted of a loopful (5 sq. mm.) of an 0.9 per cent saline suspension of cells obtained after 18 hours of incubation. The density of these suspensions was standardized by means of a Klett-Summerson photometer with a #66 filter.

The agar surface was streaked in the conventional manner used to obtain the greatest number of isolated colonies. The amount of growth was recorded arbitrarily as zero to four plus on the basis of the number and size of the colonies at the most

---

* Fellow of the Dazian Foundation for Medical Research.
** Assistant Professor of Biochemistry.
† This research was aided by a grant from the Medical Fluid Research Fund of the Yale University School of Medicine and is to be submitted in partial fulfillment of the requirements for the degree of Doctor of Medicine by one author (P. J. G.).
Received for publication January 9, 1957.

isolated portion of the agar surface. The medium of Gould,[5] modified only by the addition of 2.0 mg. per cent of glutathione, was employed for most of the growth factor determinations and this medium is hereafter referred to in the text as "basal agar." All cultures were incubated at 37°.

The following components (in grams) comprised the medium devised for the clinical studies: technical casamino acids, 15*; starch, 1.5; glucose, 1.5; KH_2PO_4, 1; $Na_2HPO_4 \cdot H_2O$, 2.8; $MgSO_4 \cdot 7H_2O$, 0.45; yeast extract, 7.5*; agar, 17, and distilled water, 1,000 ml. The medium was adjusted to pH 7.4 with 10 per cent Na_2CO_3, autoclaved for 10 minutes at 10 pounds pressure (113°), cooled quickly and, to avoid reheating, immediately poured into glass or plastic petri dishes. After solidification, each plate was hermetically sealed with a rubber petri dish seal and then stored at 3-5°. In the preparation and use of this medium it should be noted that, for optimal growth, both reheating the sterile medium and the addition of larger amounts of yeast extract are to be avoided.

EXPERIMENTAL

Preliminary studies using direct transfers of microorganisms from stock cultures showed that both old and recently isolated strains of *N. gonorrhoeae* grew under CO_2 on basal agar. Growth did not occur in air unless the medium contained a supplement of hypoxanthine, uracil, and oxaloacetate. However, this supplemented agar supported only slight growth for the same time period (24 hours) in which moderately heavy growth was obtained under CO_2; at the end of 42 hours, heavy growth also was obtained in air.

It seemed likely that, when the organism is grown in air, the decomposition of oxaloacetic acid in the alkaline medium produces the CO_2 necessary for growth. In general, one of the effects of CO_2 on the growth of bacteria appears to be concerned with a reduction in the lag period of growth. Therefore, increasing concentrations of oxaloacetic acid might be expected to cause a corresponding reduction in the lag phase of growth in *N. gonorrhoeae*. Oxaloacetic acid was cautiously neutralized with Na_2CO_3, the solution sterilized by filtration through a Swinny filter (Becton-Dickinson Company), and employed in concentrations varying from 5 to 80 mg. per 20 ml. of basal agar. From Table 1 it may be seen that when the sole addition to the basal medium is oxaloacetic acid, there is no observable growth in air. However, in the presence of hypoxanthine and uracil, which promote growth in air, the addition of oxaloacetate increases the amount of growth. The amounts of hypoxanthine and uracil employed were those found previously[7] to be optimal for the growth of *N. gonorrhoeae*. The addition of 0.05 gm. of yeast extract (previously sterilized at 113° for 10 minutes) to

* Generously supplied by Difco Laboratories, Detroit, Michigan.

the completely supplemented basal agar not only caused a significant reduction in the lag period but also increased the amount of growth in air.

If the function of oxaloacetate is to supply small amounts of CO_2 which is lost when the plate is exposed to air, then growth should occur within 24 hours in sealed basal agar plates containing only oxaloacetic acid but should not occur in plates containing only hypoxanthine and uracil. Furthermore, if the free CO_2 were to be absorbed by NaOH (in sealed Spray anaerobic

TABLE 1. EFFECTS OF OXALOACETATE AND YEAST EXTRACT ON GROWTH OF *Neisseria gonorrhoeae*

	Air		*Carbon dioxide*	
	Hours of incubation			
Basal agar	*24*	*42*	*24*	*42*
20 ml. Supplemented with:				
oxaloacetic acid (5,10, 20,40,80 mg.)	—	—	2+	3+
hypoxanthine (70 μg.) and uracil (80 μg.)	—	1+	2+	3+
hypoxanthine (70 μg.) uracil (80 μg.) oxaloacetic acid (5 mg.)	—	3+	2+	3+
hypoxanthine (70 μg.) uracil (80 μg.) oxaloacetic acid (5 mg.) yeast extract (0.05 gm.)	3+	4+	3+	4+
Yeast extract only (0.05 gm.)	3+	4+	3+	4+

Density of suspension = 25 Klett units.

petri dishes), growth would not be expected in the presence of either oxaloacetic acid alone or combined with hypoxanthine and uracil. The data presented in Table 2 are in accord with this hypothesis.

The importance of the total concentration of CO_2 on the growth of *N. gonorrhoeae* can be inferred from the data in Table 2 since in the presence of oxaloacetate growth occurred sooner in hermetically sealed petri dishes (12 mm. deep) than in sealed Spray dishes (50 mm. deep) containing water instead of NaOH. This would be expected if an effect of oxaloacetate is to contribute to a suitable concentration of CO_2. The marked effect of yeast extract demonstrated in Table 2 also was noted when even lighter inocula were used, further indicating the need for factor(s) in addition to

hypoxanthine and uracil. In view of the suggested relationship of biotin to CO_2 fixation and oxaloacetic acid synthesis, and because of the presence of biotin in yeast extract, this material was tested for its effect on growth. In these experiments, vitamin-free casamino acids (Difco) replaced the usual technical casamino acid component. The addition of 10 μg. of biotin to basal

TABLE 2. GROWTH OF *Neisseria gonorrhoeae* IN HERMETICALY SEALED AND UNSEALED DISHES IN PRESENCE AND ABSENCE OF NaOH

	Standard petri dishes				*Sealed Spray-anaerobic dishes*			
	not sealed		*sealed*		*over NaOH*	*over H_2O*		
Basal agar (20 ml.)	*18 hrs.*	*22 hrs.*	*18 hrs.*	*22 hrs.*	*48 hrs.*	*18 hrs.*	*22 hrs.*	*48 hrs.*
oxaloacetic acid (5 mg.)	—	—	—	+(s)**	—	—	—	±
hypoxanthine (70 μg.) uracil (80 μg.)	—	—	—	—	—	—	—	—
hypoxanthine (70 μg.) uracil (80 μg.) oxaloacetic acid (5 mg.)	—	+(s)	+	2+	—	—	—	+
hypoxanthine (70 μg.) uracil (80 μg.) oxaloacetic acid (5 mg.) yeast extract (0.05 gm.)	2+	3+	3+	4+	—	2+	3+	4+
Basal agar plus 0.05 gm. yeast extract*	3+							

Density of suspension = 19 Klett units.
* Control plate maintained in the presence of 10 per cent CO_2.
** Slight growth.

agar medium containing hypoxanthine, uracil, and oxaloacetate reduced the lag period noted in air (Table 3).

Welton *et al.*[11] have described the use of indoleacetic acid as a component of a defined medium for the growth of *N. gonorrhoeae*. Since tryptophan is destroyed in large part by acid hydrolysis, it seemed possible that the yeast stimulation noted in basal agar containing casamino acids prepared by acid hydrolysis might also reflect a requirement for tryptophan. This substance was found to stimulate growth and, furthermore, complemented the effect of biotin (Table 3).

The presence of still another stimulating factor(s) is evident since heavier growth occurred in all cases when yeast extract was added to vitamin-free casamino acid agar alone or together with optimal amounts of hypoxanthine, uracil, oxaloacetic acid, tryptophan, and biotin. The effect of yeast extract employed alone could not be accounted for solely on the

TABLE 3. REDUCTION OF GROWTH LAG PERIOD IN *Neisseria gonorrhoeae* BY BIOTIN AND TRYPTOPHAN

Vitamin-free casamino acid agar plus vitamin mix, hypoxanthine, uracil, oxaloacetate†*	*Growth in air hours*	
additions/20 ml. agar	*24*	*42*
none	—	+(s)**
biotin (10 μg.)	+	2+
L-tryptophan (0.6 mg.)	+	2+
biotin (10 μg.) L-tryptophan (0.6 mg.)	3+	4+
biotin (10 μg.) L-tryptophan (0.6 mg.) yeast extract (0.05 gm.)	4+	
yeast extract (0.05 gm.)	4+	

Density of suspension = 17 Klett units.

* Vitamin mix (in mg.): pyridoxine HCl, 3.0; thiamine HCl, 4.5; riboflavin, 2.0; nicotinic acid, 26; water, 10 ml., pH 6.9. Autoclaved at 113° for 10 minutes. Employed at 0.01 ml./20 ml. agar.

** Slight growth.

† Additions per 20 ml. agar: hypoxanthine, 70 μg., uracil, 80 μg., and oxaloacetate, 5 mg.

basis of the content of biotin and tryptophan. The concentration of yeast extract used throughout these experiments is of paramount importance since too much yeast extract decreases or prevents growth presumably because of the presence of an inhibitory substance.

In another series of experiments the growth of *N. gonorrhoeae* (stock strains) from very dilute inocula (density of 5 Klett units) on hermetically sealed, basal agar plates supplemented with yeast extract was found to be superior to that obtained with Mueller-Hinton agar under CO_2. On the basis of these observations a possible clinical application was suggested.

A comparative clinical evaluation was made of the growth and isolation of *N. gonorrhoeae* from materials obtained from both male and female patients. Growth in 24 hours on the yeast-agar medium (see methods) by this technique was compared with that obtained using the standard chocolate agar-CO_2 method. Data from 83 unselected, consecutive cases are presented in Table 4. Without exception, the new method duplicated the results obtained with the standard technique; in many instances it yielded more abundant growth and in three cases a positive finding was obtained, whereas negative cultures resulted through the use of the standard technique.

TABLE 4. COMPARISON OF STANDARD METHODS WITH PROPOSED AGAR-TECHNIQUE IN THE CLINICAL ISOLATION OF *N. gonorrhoeae*

	*Number of cases**	
Readings	*Chocolate agar carbon dioxide*	*Yeast-agar hermetically sealed*
Negative	48	45
Positive	35	38

* Nineteen from female patients with the following distribution: positive, 3; negative, 16.

DISCUSSION

It is generally accepted that essentially all microorganisms require a certain minimal concentration of CO_2 before reproduction occurs, the required level of CO_2 varying with the microorganism. This is particularly evident among certain members of the genera *Brucella* and *Neisseria*. Various reports (Walker,[20] Winslow *et al.*,[22] Gladstone *et al.*,[4] Tuttle and Scherp[19]) have demonstrated a relationship between CO_2 concentration and the lag phase of growth, and the concept is that cell division occurs only after a certain critical level of CO_2 concentration has been reached. The attainment of such concentrations is determined by factors regulating the rate of release of small amounts of CO_2 from substances present in the medium, combination of CO_2 with constituents in the medium, loss through diffusion, and the amount produced by the respiration of the microorganism.

Some controversy exists as to the actual replacement of CO_2 by known growth factors. Ajl and Werkman[1] reported that certain components of the citric acid cycle met the CO_2 requirement of *Escherichia coli* whereas Lwoff and Monod[10, 11] contended that CO_2 per se is essential. Support for the latter view has been provided by Gutterman and Knight,[8] who suggest that oxaloacetic acid satisfies the CO_2 requirement of *Penicillium chrysogenum*

through decomposition and the slow release of CO_2, and by Tuttle and Scherp,[19] who described various combinations which partially replace the requirement for supplemental CO_2 but failed to eliminate this need completely. Furthermore, Newton *et al.*[14,16] and Newton and Wilson[15] showed that the addition to the medium of some of the products of $C^{14}O_2$ fixation (pyrimidines) in *Brucella abortis* did not completely satisfy the CO_2 requirement of this organism. The persistent requirement by *Treponema pallidum*, strain 69, for CO_2 is another example of the phenomenon (Steinman *et al.*[18]).

In the present report the effect of oxaloacetate on the growth of *N. gonorrhoeae* has been shown to be related to the requirement of this organism for CO_2. These data lend additional support to the proposal that CO_2 is needed for the growth of this microorganism and that no growth will occur under conditions where CO_2 is efficiently and rapidly removed.

In a variety of bacteria, biotin has been shown to be necessary in the decarboxylation of oxaloacetic acid to pyruvate and CO_2 (Lardy *et al.*,[9] Shive and Rogers[17]). Biotin has also been implicated in purine biosynthesis.[18] The participation of biotin in CO_2 fixation by certain microorganisms has been described by Lardy *et al.*[9] and Blanchard *et al.*[5] The latter authors suggested that, in their experiments, biotin does not function directly as a cofactor in the carboxylation reactions of *Lactobacillus arabinosus* but may be a component in those reactions leading to the synthesis of enzymes which control the fixation of CO_2.

Against this background, the demonstration in the present report of the absolute need for CO_2 by *N. gonorrhoeae* and the finding of the stimulatory effect of biotin suggests an interrelationship between these substances. This problem currently is under investigation. The further stimulation of growth by yeast extract added to basal agar supplemented with hypoxanthine, uracil, oxaloacetate, biotin, and tryptophan reflects the participation of still another factor(s), the nature of which is also under study.

The recognition of the need for suitable levels of CO_2 as such in the growth of *N. gonorrhoeae* and the ability of yeast extract to substitute for these factors suggested the use of a technique for the isolation of *N. gonorrhoeae* from clinical specimens which employs a medium hermetically sealed in petri dishes. This new technique possesses the following advantages: it allows more growth in a shorter period of time compared with some of the media in standard use, it is convenient to prepare, economical, stable on storage, and, because of the rubber seal, can be stored for prolonged periods without contamination or loss of moisture. Furthermore, the method eliminates the need for blood or serum, as well as the use of cumbersome CO_2

jar devices. In view of these advantages, the use of this technique in the isolation of *N. gonorrhoeae* from clinical materials is proposed.

SUMMARY

1. It has been demonstrated that a rôle of oxaloacetate in the growth of *N. gonorrhoeae* in air on basal agar supplemented with hypoxanthine and uracil is to supply CO_2. Furthermore, under these conditions the absolute need for CO_2 has been shown.

2. Using a light inoculum and a supplemented vitamin-free casamino acid agar-medium, the need for biotin and tryptophan was established. An additional factor(s) in yeast, which stimulates the growth of this microorganism, was also demonstrated.

3. A simplified agar medium used in combination with hermetically sealed petri dishes for the isolation of *N. gonorrhoeae* from clinical specimens is described and the advantages of such a technique are discussed.

ACKNOWLEDGMENTS

We wish to thank Dr. Clement Batelli of the Bureau of Laboratories, New Haven Department of Public Health, New Haven, Connecticut, for his cooperation and Miss Katherine Connelly for her assistance in performing the comparative clinical evaluation of the technique described in this report.

REFERENCES

1. Ajl, S. J. and Werkman, C. H.: Replacement of CO_2 in heterotrophic metabolism. Arch. Biochem., 1948, *19,* 483-492.
2. Blanchard, M. L., Korkes, S., del Campillo, A., and Ochoa, S.: Function of biotin in the metabolism of *Lactobacillus arabinosus.* J. biol. Chem., 1950, *187,* 875-890.
3. Gitterman, C. O. and Knight, S. G.: Carbon dioxide fixation into amino acids of Penicillium chrysogenum. *J. Bact.,* 1952, *64,* 223-231.
4. Gladstone, G. P., Fildes, P., and Richardson, G. M.: Carbon dioxide as an essential factor in the growth of bacteria. Brit. J. exp.. Path., 1935, *16,* 335-348.
5. Gould, R. G.: Glutathione as an essential growth factor for certain strains of *Neisseria gonorrhoeae.* J. biol. Chem., 1944, *153,* 143-150.
6. Griffin, P. J. and Racker, E.: Replacement of the carbon dioxide requirement of *Neisseria gonorrhoeae* by hypoxanthine, uracil, and oxaloacetate. Bact. Proc., 1955, 114-115.
7. Griffin, P. J. and Racker, E.: The carbon dioxide requirement of *Neisseria gonorrhoeae.* J. Bact., 1956, *71,* 717-721.
8. Lardy, H. A., Potter, R. L., and Elvehjem, C. A.: The role of biotin in bicarbonate utilization by bacteria. J. biol. Chem., 1947, *169,* 451-452.
9. Lardy, H. A., Potter, R. L., and Burris, R. H.: Metabolic functions of biotin. I. The role of biotin in bicarbonate utilization by *Lactobacillus arabinosus* studied with C^{14}. J. biol. Chem., 1949, *179,* 721-731.
10. Lwoff, A. and Monod, J.: Essai d'analyse du rôle de l'anhydride carbonique dans la croissance microbienne. *Ann. Inst. Pasteur,* 1947, *73,* 323-347.

11. Lwoff, A. and Monod, J.: The problem of heterocarboxylic metabolites. Arch. Biochem., 1949, *22,* 482-483.
12. Moat, A. G. and Wilkins, C. N.: Biotin in purine synthesis. Fed. Proc., 1956, *15,* 605.
13. Mueller, J. H. and Hinton, J.: A protein-free medium for primary isolation of the gonococcus and meningococcus. Proc. Soc. exp. Biol., 1941, *48,* 330-333.
14. Newton, J. W., Marr, A. G., and Wilson, J. B.: Fixation of $C^{14}O_2$ into nucleic acid constituents by *Brucella abortis.* J. Bact., 1954, *67,* 233-236.
15. Newton, J. W. and Wilson, J. B.: CO_2 requirements and nucleic acid synthesis by *Brucella abortus.* J. Bact., 1954, *68,* 74-76.
16. Newton, J. W., Wilson, J. B., and Wilson, P. W.: Nucleotides and nucleic acid synthesis by *Brucella abortus.* J. Bact., 1955, *69,* 677-681.
17. Shive, W. and Rogers, L. L.: Involvement of biotin in the biosynthesis of oxaloacetic and α-ketoglutaric acids. J. biol. Chem., 1947, *169,* 453-454.
18. Steinman, H. G., Oyama, V. I., and Schulze, H. O.: Carbon dioxide, cocarboxylase, citrovorum factor, and coenzyme A, as essential growth factors for a saprophytic treponeme (S-69). J. biol. Chem., 1954, *211,* 327-335.
19. Tuttle, D. M. and Scherp, H. W.: Studies on the carbon dioxide requirement of *Neisseria meningitidis.* J. Bact., 1952, *64,* 171-182.
20. Walker, H. H.: Carbon dioxide as a factor affecting lag in bacterial growth. Science, 1932, *76,* 602-604.
21. Welton, J. P., Stokinger, H. E., and Carpenter, C. M.: A chemically defined medium for the cultivation of the gonococcus. Science, 1944, *99,* 372.
22. Winslow, C.-E. A., Walker, H. H., and Sutermeister, M.: The influence of aeration and of sodium chloride upon the growth curve of bacteria in various media. J. Bact., 1932, *24,* 185-208.

EVENTS

YALE MEDICAL SOCIETY

March 11, 1957

MSH AND ACTH. By Aaron B. Lerner, Associate Professor of Dermatology, Yale University School of Medicine.

In 1917 it was first observed that removal of the pituitary glands of tadpoles resulted in a lightening of the skin, while injections of exogenous pituitary extract caused increased skin pigmentation. About 1930 Zondek found that this skin-darkening principle was produced by the pars intermedia, and hence he proposed the name Intermedin for it. The pituitary skin-darkening hormone is now called melanocyte stimulating hormone (MSH) to indicate its trophic function.

Attempts to purify extracts from the pars intermedia of hogs in collaborawith T. H. Lee resulted in an homogenous compound with considerable melanocyte stimulating activity. Workers elsewhere who were attempting to do the same thing by different techniques also isolated a homogenous compound which was different in its electrophoretic properties, but which also displayed MSH activity. Bioassay of these two compounds showed that the one isolated by the Yale group was the more active of the two, and the names alpha and beta MSH were given to the more and less active compounds respectively.

Following the determination of the structure of beta MSH by other workers, the author assisted J. I. Harris in the study of the structure of alpha MSH. The experimental work was carried out with a few milligrams of alpha MSH which seemed to be homogenous when subjected to electrophoresis. Hydrolysis of the compound yielded twelve different amino acids, with serine appearing in twice as great molar quantities as any of the others. Attempts to identify the C and N terminal amino acids indicated that the carboxyl and amino groups of these amino acids, respectively, were blocked. The specific actions of trypsin and chymotrypsin were used to obtain shorter fragments of the thirteen amino acid chain, and to determine the position of certain amino acids in the compound. Having located these amino acids, acid hydrolysis followed by analysis of the resulting peptides was employed to determine the location of the other amino acids in the chain. The following sequence of amino acid residues was found:

Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Try-Gly-Lys-Pro-Val.

An amide group was determined to be the blocking group on the valine residue. The N terminal blocking group is still unknown.

It is interesting that these thirteen amino acids in the sequence indicated correspond exactly to the first thirteen amino acid residues of ACTH. Moreover, a chain of seven amino acids included in the eighteen amino acid chain of beta MSH corresponds with the arrangement of seven of the amino acids in alpha MSH.

DAVID W.E. SMITH

PLASMA 17-KETOSTEROIDS. By Lytt I. Gardner, Professor of Pediatrics, Yale University School of Medicine.

Methods for the determination and study of plasma 17-ketosteroids have been developed and have shown the relationship between various normal

and pathological conditions and plasma 17-ketosteroid levels. The procedure for isolation of these compounds consists of acid hydrolysis of plasma samples, successive extraction with ether, alkali, and hexane, chromatographic separation on Florisil, and treatment of the residue with *m*-dinitro benzene.

The average plasma 17-ketosteroid level for adults was found to be 61 μg/100 ml; the average for the newborn was somewhat higher, about 80 μg/100 ml. This high level in the newborn declines after two to three days.

The plasma 17-ketosteroid level was significantly changed in several disease states. In subjects with pure virilizing adrenal tumors, or with untreated congenital adrenal hyperplasia, the level averaged about 300μg/100 ml or higher. The level is also elevated in the rare neoplastic Cushing's syndrome. If a subject with congenital adrenal hyperplasia is given cortisone, which satisfies the inherent metabolic error of this disease, the plasma level of 17-ketosteroids drops to a normal value. Benemid, a tubular blocking agent, causes an increase in plasma 17-ketosteroids.

A study was made of the plasma 17-ketosteroid levels of expectant mothers during various stages of pregnancy and labor, and during lactation. Since the level is higher in umbilical plasma than in maternal plasma, it is concluded that these materials probably originate in the fetal circulation at this time. A sharp increase in these levels occurs during the few hours of labor just prior to delivery; the level decreases sharply to a very low value following delivery and rises slowly to a normal value during lactation. The sharp increase at the time of delivery is thought to be due to back diffusion associated with uterine contractions.

Other workers have shown that all of the 17-ketosteroids could be accounted for as dehydroepiandrosterone and androsterone. In one case a premature baby showed a plasma 17-ketosteroid level of over 500 μg/100 ml during the first two days of life.

This new technique for the determination of plasma 17-ketosteroids will undoubtedly lead to a better understanding of the relationship between these compounds and the various physiological and pathological processes in which they are involved.

MALIN R. DOLLINGER

NEUROLOGICAL STUDY UNIT

March 12, 1957

SPINAL REFLEX PATTERNS. By John W. Magladery, Associate Professor of Neurology, Johns Hopkins Hospital and Medical School, Baltimore, Maryland.

The method by which Paul Hoffman investigated tendon jerk reflexes employed shocks to skin overlying a nerve trunk which were of a magnitude to cause afferent stimulation without stimulation of efferent fibers. The muscle response, recorded electrically as sharp spike action potentials, was similar to that elicited by a tendon tap. Electrical overstimulation caused a reduction in the magnitude of the response, indicating the induction of antidromic impulses and a refractory state in efferent fibers. These reflexes, which shall

be referred to as H reflexes in deference to Hoffman, have the following characteristics. (1) The central delay is compatible with passage through a single synapse when the excitatory stimulus is of low intensity. (2) The most excitable afferents have lower excitation thresholds and higher conduction velocities than the motor axones. (3) The afferent limb is more susceptible to functional interruption than is the efferent limb. (4) Monosynaptic discharge occurs only when the afferent stimulus arrives at the cord via the fibers of lowest threshold. With larger afferent volleys, evidence of internuncial activity appears. (5) H reflexes can be elicited from large muscles of both flexor and extensor groups only. (6) Profound suppression of H reflexes is found to occur after the slightest jar of muscle but not after painful stimulation of the overlying skin. (7) Electrical stimulation reveals a facilitation of the second of consecutive stimuli when they occur within 25 msec. This is succeeded by a depression lasting several hundred msec. Mechanical stimulation lacks an elicitible facilitatory phase. Root recordings show these effects to be central in origin.

Patients with upper motor neuron lesions of the cord or lower brain stem show a lowered afferent threshold and enhanced reflex discharge. Moreover, reflexes can be recorded from muscles in which they are normally unattainable. Latency of reflexes elicited by liminal shock is 4-6 msec. longer than the normal 32-34 msec., and the shape of the recorded response is polysynaptic. These findings have been corroborated in experiments using cats. The clinical status of the patient, however, does not correlate well with the physiological findings. This fact implies mechanisms outside the myotatic arcs themselves.

N. R. M.

ZOOLOGICAL JOURNAL CLUB

April 15, 1957

THE RÔLE OF CELL DEATHS IN MORPHOGENESIS OF AVIAN LIMBS. By John W. Saunders, Department of Biology, Marquette University, Milwaukee, Wisconsin.

Cell degeneration and death play a considerable and perhaps necessary part in chick limb morphogenesis. Waves of cell dissolution act to carve wing, leg, and digital contours. An area of cell death is also found on the somites. Nile blue sulfate selectively dyes these degenerating cells.

A leg bud grafted to the body wall shows the degeneration at the same time as an unoperated control. Therefore, degeneration takes place as a result of localized factors. If the area of future cell death is excised after stage 19, there is no cell degeneration at the site of the operation, but if excised before stage 19, the cells die. The capacity to degenerate is therefore restricted to certain cells by stage 19, but the death rôle may be taken over by other cells before that time. When cells from the area of degeneration at the posterior angle of the wing are grafted to the dorsal surface of the wing bud prior to stage 22, there is no degeneration; if the graft is made early in stage 23, cell death takes place in the graft. Cells grafted to a somite as early

as stage 17 later degenerate, indicating that the tissue environment of the somite is more favorable to degeneration than the dorsal surface of the wing. If the limb is deprived of the degenerative cell area, it apparently undergoes normal morphogenesis. The exact rôle of cell death, however, will not be determinable until an experiment can be devised in which cell degeneration can be stopped *in situ* and the effect be observed.

G. E. W., 3D

BERTRAM ROBERTS MEMORIAL LECTURE

May 2, 1957

INTRODUCING A PSYCHIATRIC CLINIC INTO A COMMUNITY. By Alexander Leighton, Professor of Social Psychiatry, Cornell University, Ithaca, New York.

Psychiatric clinics in communities under 250,000 will probably become more prevalent if the present wave of interest in psychiatry keeps up. The experimental clinic set up by Cornell University and the Nova Scotia Department of Public Health six years ago at Bristol,* a town of 3,000 on the Atlantic coast of Canada, exemplifies the problems and the evolution of such a clinic from its initial impact through its incorporation into the community at large.

The establishment of such a clinic initially involves the planning of immediate and long-term goals. Thus, as the community becomes aware of the clinic and perceives its potentialities, the clinic can at all times maintain a working relationship and become firmly accepted in professional and lay circles. The clinic must be prepared to be accepted on the community's terms until understanding and confidence have developed. Identification with previously established institutions which are relatively nonpartisan, such as the local medical society, is, at the outset, of great importance. As the clinic develops, sentiments crystallize in all quarters. However, the majority of the community remain indifferent, and positive or negative attitudes will develop depending on the professional and social record which the members of the clinic make with the passage of time. The importance of being known and accepted as a human being in a small community is not to be undersetimated and provides special problems to the practice of psychiatry.

As firmer roots develop, the general practitioners begin to refer cases earlier, with better selection, and become more involved in the psychiatric care of the mentally ill and may even do some therapy themselves. The question of the rôle of the general practitioner, the clergy, the teachers, and the hospital comes up and must be worked out as the clinic becomes more active. With greater awareness of its needs, the community begins to ask more of the clinic in frequency and range of cases. The potentiality of the clinic is slowly realized and fulfilled so that eventually cases are seen early,

* A code name to protect the privacy of the area under study.

there is a good follow-up and after-care program, effective mental health programs are instituted, and investigation of mental illness in the community epidemiologically, genetically, and pathologically can be carried out.

R. N.

RALPH HILL THOMAS LECTURE

May 20, 1957

THE GROWTH OF SINGLE CELLS. By J. Murdock Mitchison, Zoology Department, University of Edinburgh, Scotland.

A measure of the growth of a single cell may be obtained by finding the increase in dry matter. Using an interference microscope with the cell mounted in an aperture, by measuring the retardation with the cell in place and then removed, the total retardation by the cell (a function of dry matter) may be calculated without measuring the size of the object. The method is accurate (i.e., repeatable) to 5×10^{-14} grams which is the dry mass of 1 μ^3 of cytoplasm. Fission yeast, *Schizosaccharomyces pombe* fulfils the requirements for an experimental cell: (1) regular shape (cylinder), (2) reasonable size, (3) rapid growth, (4) grows in a sterile medium, (5) does not move while using the substrate. Growth takes place only in length at the end away from the preceding division and the cell divides with a plant-like central plate.

The cell was mounted in glue; retardation was measured and a photograph taken to record size every 7½ minutes. Dry mass growth rate curves consist of a rising straight line, a break at division, then a steeper straight line. Volume (length in this case since the cell grows only in one plane) grows at a higher rate, reaches a plateau at division, and rises again after division. The volume normally growing at one end only probably stops increasing while the cell plate is formed in the center. A possible mechanism for division may be that as the concentration within the cell increases, hydrostatic pressure between the two cell plates blows them apart, since, when the two cells separate, they round out. There is no real evidence confirming this view, and division appears to take place by pinching off. A model consisting of synthetic centers or particles controlling growth is proposed. These remain constant in number until just before division when they double and separate at "mitosis." This would account for the observed sudden doubling in synthetic rate after division. These findings are in agreement with those of Zeuthen on respiratory rate in *Tetrahymena* but differ from those of Prescott on *Amoeba* in which the growth rate is not constant but rises exponentially. This may be due to a decreasing rate of feeding and rounding up before division in *Amoeba*. Budding yeast growth rates follow an S curve for volume, but the mass growth is linear.

Further work is intended with other cells including *Prototheca* and bacteria; electron microscope studies of mitochondrial and microsomal activity; and growth rates with changed environments in synchronous cultures.

G. E. W., 3D

RADIATION THERAPY SEMINAR

May 23, 1957

SUPERVOLTAGE RADIATION THERAPY WITH BETATRON. By William B. Seaman, Professor and Director, Department of Radiology, College of Physicians and Surgeons, Columbia University, New York.

The principal features of the Betatron supervoltage emission which recommend it over conventional x-ray are: (1) its ability to deliver a high percentage of a total dose to deeply situated tissues, (2) its property of sparing the skin overlying the target, even at high dosages, (3) its bone-sparing property (absence of differential absorption by bone), and (4) its exceptionally low side-scatter. The latter property recommends the Betatron for treatment of neoplasms adjacent to structures such as the eye or spinal cord, to which the side-scatter of conventional x-radiation would be damaging. Bone sparing is especially advantageous in radiation of the face and skull.

Supervoltage radiation therapy works no magic. It is beset with the limitations and problems of conventional x-radiation therapy. The misconceptions that patients with disseminated carcinoma or with radio-resistant tumors are good candidates for supervoltage therapy have led to therapeutic failures in these ultimately hopeless cases.

In treatment of local visceral cancers including brain tumors supervoltage therapy has yielded promising results with a low incidence of radiation sickness and minimal damage to adjacent normal tissues. The present experience indicates that the "biological effectiveness" of x-rays with this energy is similar to that of the 250 kv range, instead of 20-40 per cent lower. Dosages and normal tissue tolerances are becoming better established as experience with the Betatron accumulates.

R. P. A.

BOOK REVIEWS

THE COLLECTED PAPERS OF PAUL EHRLICH. Compiled and edited by F. Himmelweit, with the assistance of Martha Marquardt, and under the editorial direction of Sir Henry Dale. Volume I. *Histology, Biochemistry and Pathology.* London and New York, Pergamon Press, 1956. viii, 653 pp. $85 (4 vols.).

This important volume is the first of four; the second will deal with Ehrlich's work on immunology and cancer, the third with his studies in chemotherapy, and the fourth, in addition to a bibliography and indexes, will contain tributes by Ehrlich to his teachers, supporters, and colleagues. The papers collected in the first volume describe work done by Ehrlich during the period 1877-1890, as a university student, as a young hospital physician, and as a free-lance laboratory worker. Although his studies on immune reactions and on chemotherapy, which brought Ehrlich world-wide fame, came after 1890, the earlier work was no less important, since it included his research on the use of aniline dyes for hematological and bacteriological staining, for the study of biochemical reactions, and for the examination of pathological tissues and fluids.

The exceptional imagination that characterized Ehrlich's scientific thought is evident throughout this volume, and is nowhere clearer than in his previously unpublished doctoral dissertation of 1878: "Beiträge zur Theorie und Praxis der histologischen Färbung" (an English translation is also included). Written when Ehrlich was 24 years old, the dissertation contains the germs of many of the ideas he was to pursue until his death in 1915. For the student of the history of science, this dissertation has special interest; it underlines Ehrlich's rôle in the application, to biological studies, of the experience gained by the dyers of textiles in their use of the newly developed aniline dyes. By 1877, Germany had initiated the development of an active dyestuff industry and also took the lead in the synthesis of new dyes.

After his doctorate, Ehrlich concentrated on the development of improved procedures for the staining of tissues, of blood cells, and of bacteria. From these studies, he moved to an examination of the capacity of biological systems to reduce dyes and to re-oxidize reduced dyes in the presence of oxygen. This volume contains Ehrlich's memorable paper of 1885 on "Das Sauerstoff-Bedürfnis des Organismus" (also with an English translation), which foreshadows ideas that emerged more clearly from the later work of Battelli and Stern (*ca.* 1910), of Thunberg (*ca.* 1920), and of Keilin (*ca.* 1926). The influence that Ehrlich's early studies had on his followers, especially on Leonor Michaelis (1875-1949), is striking.

The volume is made even more valuable by an affectionate introduction by Sir Henry Dale. The format is most attractive, and the editing appears to have been done with exemplary care. The publication of all four volumes is made possible by the enlightened philanthropy of The Wellcome Trust. The editors, the sponsors, and the publishers are to be congratulated on having given to the world of science this splendid addition to its historical literature, and so appropriate a tribute to the greatness of Paul Ehrlich.

JOSEPH S. FRUTON

DISEASES OF THE HEART AND CIRCULATION. By Paul Wood. Philadelphia, J. B. Lippincott Company, 1956. 2d ed. 934 pp. $15.00.

This book represents an expanded and drastically revised version of an earlier (1950) edition which gained wide recognition. The author, who is a leading British cardiologist, has compressed his extensive clinical experience into a well-written, informative account which is a model of conciseness and clarity. He has organized his material according to physiological and etiological considerations, rather than according to morbid anatomy. This arrangement allows the author to explore his deep interest and special experience in rheumatic and congenital heart disease and the special methods, such as cardiac catheterization, related thereto. These chapters are outstanding. On the other hand, this technique also leads to somewhat abbreviated treatment of some of the more unusual or anatomically oriented aspects of heart disease such as myocarditis, pericarditis, and subacute bacterial endocarditis. Peripheral vascular disease is not covered, the term "circulation" in the title having a physiological connotation. There is, for a general textbook, an unusually good section on radiographic diagnosis, but only the usual abbreviated section on electrocardiography. Every chapter contains repeated astute, concise, and practical clinical observations, which (in the words of the author) "maintain a balance between bedside medicine and special tests." Plentiful illustrations further clarify an already lucid text. References are abundant and the index is not only quite complete, but also has been arranged for use in differential diagnosis.

This book is primarily intended for graduates particularly interested in clinical cardiology, but it is of wide appeal to students and general internists as well.

ALLAN V. N. GOODYER

CONGENITAL ANOMALIES OF THE VISCERA: THEIR EMBRYOLOGICAL BASIS. By J. Lewis Bremer. Cambridge, Harvard University Press, 1957. 187 pp. $5.00.

Congenital Anomalies of the Viscera is a superb book. It is the only modern textbook presentation of this subject and is presented in orderly delineation with great clarity and economy of text and illustration. The author intends that it supplement the medical school course in normal embryology as a text for "the graduate in medicine who is starting an internship or residency in pediatrics, and for one already engaged in the practice of this branch of medicine." This breadth of scope makes its pedagogical design interesting, for the necessarily traditional "review that is in order" can be accomplished without obfuscation of the real subject matter by extraneous verbiage on background material. Were it not for the absence of chapters on cleavage, fetal membranes, etc., the book would be adequate as an embryology text. Just as pathology tends to make normal anatomy, histology, and physiology better understood, so, too, is a knowledge of normal development enhanced by a comprehension of the dynamics of malformation. Moreover, a sympathy for the general types of events that lead to anomalies is cultivated in order that "From the knowledge of these

developmental sequences new types of abnormality, either hitherto undescribed or published merely as rare and unexplained findings, can be understood and traced to their embryological sources."

The discussion of anomalies is in terms of aberrant growth and disturbances of the growth sequence per se. Thus, although the preface states that "The ultimate cause of these slight local changes of growth rate may be chemical or hormonal or thermal," there is no substantiation to be found in the body of the text. Despite this absence of primum mobile, the secondary and tertiary spheres of influence (clinical, physiological, etc.) of the anomalies are represented in factual, speculative, and sometimes anecdotal discussion. Much is of diagnostic importance. In some instances, e.g. that of traction diverticula of the trachea (which Bremer considers to be formed in a manner similar to the formation of a tracheo-oesophageal fistula), a groundwork is laid for possible re-evaluation of common pathological teaching.

Outside the scope of this book are tables and analyses of prevalence and incidence of the various anomalies; nor is there any discussion of medical or surgical prognosis and care or of longevity. The chapter listing is as follows: respiratory and alimentary systems; pharynx and neck; trachea, bronchi, and lungs; intestinal tract; liver and pancreas; urinary system and suprarenal gland; uterus and vagina, genital apparatus and external genitalia; heart I; heart II, septation; heart III, transposition of the great vessels; and the development of the aortic arches.

N. R. MORRIS

LIVER: STRUCTURE AND FUNCTION. By Hans Popper and Fenton Schaffner. New York, Toronto, London, The Blakiston Division, McGraw-Hill Book Company, Inc., 1957. 777 pp. $20.00.

It is appropriate that Hans Popper should be the author of a textbook on the liver. Formerly a protégé of Eppinger of Vienna, who systematized hepatic diseases by clinico-pathological correlations, Dr. Popper has collaborated with his co-author and has approached the problem of hepatic diseases fundamentally from a pathophysiological point of view. The book is a complete and well-written encyclopedia of hepatic anatomy, physiology, and pathology. That it is detailed is demonstrated by 3,735 bibliographical references exclusive of an addendum, 62 tables, and 204 well-executed figures. It is obvious that this book not only completely covers the pertinent literature but is the result of the author's unusual experience and tireless efforts. The title of the book does not do justice to its contents because it connotes a restricted topic. The physician may be amazed to find in the text abundant clinical data.

The book has been divided into seven parts. The first concerns the structure and functions of the normal liver. This thorough review must be understood before reading the remaining sections. Emphasis is placed, in particular, upon the revised structural concept, metabolic functions, and correlation of functional and histological features of the liver. Part two contains a discussion of the pathological phenomena in the hepato-biliary sys-

tem as studied from both a morphological and functional point of view. Jaundice is thoroughly reviewed and reclassified. Hepatocellular degeneration and necrosis, cholestasis, and inflammation are reviewed, following which the morphogenesis and functional aspects of fatty infiltration and cirrhosis are reconstructed and correlated with clinical features. These descriptions form the basic core of the study of liver disease. An intelligent appraisal of the patient with liver disease is impossible without knowledge of these fundamental morphological conceptions.

The third part is a clinical study of hepatic function and structure and offers a comprehensive discussion of the so-called hepatic function tests. Their physiological basis as applied to liver biopsy is emphasized. There is an outstanding description of the tests referrable to the rôle of the liver in the metabolism of protein. Part four provides the reader with a remarkably clear study of the diffuse diseases of the liver. The systematic classification of liver diseases, etiology, experimental hepatic injury, and clinical features offers informative material, particularly to the internist and gastroenterologist. This part of the text again is written from the pathological viewpoint and contains not only the author's studies but the results of other investigations. For example, the different classifications of cirrhosis, which have been always a source of contention, are reviewed and interpreted from a practical rather than academic point of view. The authors also have proposed a functional-therapeutic classification and nomenclature of cirrhosis. They suggest that cirrhosis be described and evaluated morphogenetically, etiologically, if possible, functionally, and therapeutically. Instead of employing the term "portal cirrhosis," they suggest an alternative description: malnutritional, rapidly-advancing septal (portal?) cirrhosis with moderate hepatic-cell damage and portal hypertension with esophageal varices. To some, this may initially appear superfluous but it has practical functional-therapeutic implications. Part five, focal diseases of the liver, and Part six, tumors of the liver and biliary tree, are set forth in a manner similar to Part four.

Part seven discusses the internal and external environment and the liver. It includes a comprehensive review of the relationships of the liver to other organs of the body and of the hepatic response to stress. Particularly impressive are the sections dealing with the direct and converse relationships of the liver to the endocrine organs and the kidney. This part serves its purpose in reminding the reader that the diseased liver should be regarded systemically and generally rather than focally. With this in mind, the conception of the "hepato-renal" syndrome becomes meaningless. Finally, the authors conclude their book with a synopsis of the principles of diagnosis of liver disease based on coordinated use of functional and structural observations. This has definite clinical application based upon the authors morphophysiological concept.

This text follows those of Budd, Mallory, Rolleston and McKee, Karsner, Himsworth and Spellberg as one of the classic works on the diseases of the liver. Few treatises written by pathologists will be accepted as enthusiastically by clinicians as this book.

MARTIN S. KLECKNER, JR.

RAUWOLFIA. By R. E. Woodson, Jr., H. W. Youngken, E. Schlittler, and J. A. Schneider. Boston, Toronto, Little, Brown and Co., 1957. 137 pp. $5.50.

For research workers who are interested in this important group of alkaloids, which includes the fascinating drug reserpine, this should prove to be a useful and compact reference volume because it is an attempt to collate all pertinent information available (up to about eight months ago) on the subject. The book is composed of four chapters covering four fields of knowledge on the subject, contributed by well-known authorities: Robert E. Woodson, Jr., on the botany of Rauwolfia, Heber W. Youngken on the pharmacognosy of Rauwolfia, Emil Schlittler on its chemistry, and Jurg A. Schneider on its pharmacology.

The chapter on the chemistry of the alkaloids is somewhat more satisfying than that on the pharmacology, because the chemistry is now fairly well understood and our chemical knowledge about these alkaloids near complete, having reached its culmination in the synthesis of reserpine by Woodward's group in 1956. The weakest point in our chemical knowledge, at least for now, is in the generalizations which may be drawn upon the relation between chemical constitution and reserpine-like activity. Very little on this subject appears in this book, and the reason is simply that the subject has been only superficially explored to date.

Probably the most complex problem tackled in this volume is the pharmacology of Rauwolfia. Doctor J. A. Schneider has done a creditable job in gathering together the most pertinent information and presenting it in a well-organized fashion. It is clear that he has given a great deal of thought to the problem and has brought to bear on it his own considerable investigations in the field. If one were pressed for a criticism of this chapter (and this is more in the nature of a personal desire than a criticism), one might say that it would have been interesting to know in what direction Dr. Schneider leans concerning the mechanism of action of reserpine. On the other hand, giving such an indication might easily lead to the criticism which can be made on his willingness to dismiss as minor in reserpine's mode of action the direct peripheral effects of the drug. It seems to this reviewer that a delineation of the relative importance of its peripheral actions must await more definitive investigations in this area.

NICHOLAS J. GIARMAN

LYSERGIC ACID DIETHYLAMIDE AND MESCALINE IN EXPERIMENTAL PSYCHIATRY. Louis Cholden, Ed. New York, Grune & Stratton, 1956. 85 pp. $3.00.

There are exceedingly few—if any—occasions of hunger in which the menu can suffice for the meal. This menu, a slim volume possibly raised in dignity and certainly in price by hardbacked and drug-house-backed publication, represents the Proceedings of the Round Table on LSD and Mescaline held at the 1955 meetings of the American Psychiatric Association. The gavel-enforced brevity of such a conclave is further edited for the reader who, however, might have leisure as well as curiosity. Unhappily, the interested student will find here neither comprehensive survey and summary nor ade-

quate bibliography. He will be tersely introduced to some of the salient issues and eminent researchers concerned with a fascinating era in experimental psychiatry. The earnest research spirit of the editor and contributors results in a product that, for all its inadequacies, is informative rather than sensational—a remarkable feat in a climate currently dazzled if not dazed by drugs.

Neither the effects nor novelty of drugs which alter behavior can account for contemporary interest; such agents have been long known and frequently studied. In our opinion, what is both new and compelling is contained in the misformulation, "model psychosis." More precisely, what is new is the formulation of models for psychosis, conceptual models for psychotic behavior which can be tested and proposed from a variety of frames of reference: biochemical, neurophysiological, and behavioral. The advent of "blocking agents," such as reserpine, along with the "inducing agents," such as LSD, provides the chemist with a familiar biochemical model and a behavioral system he can manipulate. Several such hypotheses are briefly reviewed in this volume, and while serotonin theories are shown to be obviously inadequate, the relevance of a naturally occurring body hormone to behavior disorder is at last experimentally broached. Similarly, the exciting possibilities in the currently developing area of the catechol amines are touched upon. While the relation of any brain or biochemical change to observed and experienced behavior remains (as ever) obscure, developments in the psychiatric field which parallel the biochemical advances are reported. Among such topics discussed are problems in the use of LSD in therapy and improved understanding of management problems on the part of therapists who took the drug. Neurophysiological techniques and the rôle of reticular systems are also sketchily represented here; the research of the experimental psychologist unfortunately is not.

The descriptions of experimental psychosis are valuable because of their compactness, but the unusual feature of this introduction to experimental psychiatry is a viewpoint that questions the implication that drug-induced hallucinations are necessarily psychotic or unreal. LSD and mescaline do not compel psychosis in all subjects but may produce visionary experience. "What reason," one contributor asks, "have we for thinking that beauty and significance are less real than ugliness or dullness?" He appears in the list of contributors as "Aldous Huxley, Man of Letters, Los Angeles, California."

DANIEL X. FREEDMAN

THE RAUWOLFIA STORY. FROM PRIMITIVE MEDICINE TO ALKALOIDAL THERAPY. Summit, New Jersey, Ciba Pharmaceutical Products, Inc., 1954. 63 pp.

The history of the Rauwolfia alkaloids is described in this soft-covered, pocketsized book. The plant containing the alkaloids was discovered in India in the seventeenth century by a French botanist who named it Rauwolfia serpentina after a German physician and explorer, Dr. Leonhard Rauwolf. It was believed that eating the root relieved man of madness; for five centuries it had been used in India to treat insanity. Modern Indian physicians used the root as a sedative and antihypertensive for two decades

before western physicians confirmed its efficacy. One of the alkaloids isolated from the plant was reserpine. Like digitalis, cocaine, and ephedrine, reserpine represents another contribution to modern therapy from folk medicine. Its story makes fascinating reading.

J. P. G.

DAIRY MICROBIOLOGY. By Edwin M. Foster, F. Eugene Nelson, Marvin L. Speck, Raymond N. Doetsch, and Joseph C. Olson, Jr. Englewood Cliffs, New Jersey, Prentice-Hall, Inc., 1957. 492 pp. $5.65.

There has long been a need in the dairy industry for a text or reference book to fill the gap between texts dealing with public health and theoretical aspects of dairy microbiology for college students and those which cater to laboratory personnel and plant operators. The present book fills this gap. In the preface the authors state that the book should provide a working reference for plant operators, field men, sanitarians, etc., as well as a text for college students. This is rather a broad classification of personnel for one text to cover. However, the authors have skillfully blended material of interest to all the above groups which heretofore has only been available to the reader in several different texts.

Some of the material is oversimplified for a college course in dairy bacteriology. Nevertheless, the authors have selected references which, if investigated by the student, will enhance his knowledge of the subject without an extensive survey of the literature. Also, this text does not overemphasize the public health aspect of dairy products, a topic which is adequately covered in other works. They may thus have endeared themselves to many people who have long felt a need for stressing the nonpathogenic aspects of dairy microbiology, many of which are everyday problems met by milk producers and dairy products processors.

Conversely, some of the material in the book is too detailed for laboratory personnel, field men, and sanitarians. However, the authors have assembled and presented it in such a manner that the reader can easily find information of concern to him and will not have to stumble through theoretical discussions on the subject.

The only fault this reviewer has found with the book is that there is some repetition between chapters. This is of little consequence to the reader interested in only a single section of the book but may be detrimental in a college course.

The text is well written and clearly presented. The authors have done extremely well in their attempt to please a great number of people in diversified sections of dairy bacteriology. It should be well received by all those in the dairy field as it is a ready reference as well as a satisfactory teaching text.

LESTER HANKIN

AGEING IN INDUSTRY. By F. LeGros Clark and Agnes C. Dunne. New York, The Philosophical Library, Inc., 1956. 146 pp. $7.50.

The authors state that their purpose "is to determine, so far as we can, what numbers of workers are physically able to continue in their various occupations beyond their mid-sixties." They have used the tables of age

and occupation published in the Census Reports for 1921, 1931, and 1951. These relate to England, Wales, and Scotland and the results are applicable principally to those areas, although there are similarities with occupational experiences in other countries. Perhaps the most striking impression gained from the book is the difficulty of getting more than a few leads from the enormous labor that went into assembling the various tables. Thirty-two occupations are studied in detail by three methods, "Moving Cohorts," "Age Group Ratios," and "Changes in the Age-structure of Occupations." In addition the authors interviewed a variety of persons with knowledge and experience of these occupations. From the trends suggested by their statistical treatment of the data and these interviews, they present a list of occupations and the estimated practical percentage survival in each occupation of men 65 years of age and older. For example, they conclude that 75 to 85 per cent of "makers of watches and clocks, workers in precious metals, and makers of musical instruments" could continue at these occupations beyond the age of 65, whereas only 5 to 15 per cent of "coal-face workers and signalmen" would probably be physically able to perform their jobs beyond 65.

The major difficulty in a study of this kind is that it cannot take much account of technological changes. Coal mine engineers might use it as an indication that more labor saving devices should be used at the coal face. But public health workers should be skeptical of attempts to generalize and apply these conclusions to localities other than England, Wales, and Scotland at times other than the present and immediate future. For reference and historical purposes, this book is of interest.

J. WISTER MEIGS

THE CARE OF THE EXPECTANT MOTHER. By Josephine Barnes. New York, Philosophical Library, 1956. 270 pp. $7.50.

One of the difficulties facing the author of a book such as this is that of striking a balance between the scope of the subject matter and the amount of discussion devoted to each subject. In its preface it is stated that this "book has been written as a practical guide for all who undertake the management of pregnancy" in Great Britain. However, the superficial attention given to much of its subject limits the usefulness of the book to a very few; in Great Britain where midwives undertake a greater proportion of prenatal care than in the United States, the purpose of the volume may be accomplished to a fuller extent.

The book is divided into three parts. Part I deals with normal pregnancy and includes an informative discussion of the maternity services in Great Britain. Abnormal pregnancy is the subject matter of Part II, and Part III describes the diseases complicating pregnancy. The text is easy to read and the illustrations, though not entirely satisfactory, are clear. Source books on preparation for childbirth and advising the mother are listed.

The principles of obstetric management in America do not coincide with many of those expressed within this book. Space limitations prohibit a complete summary, but the chief differences and omissions would include the following:

1. The section on blood groups and RH factors is limited, and the management of erythroblastosis is discussed only in relation to hydrops fetalis.

2. In breech presentations, external cephalic version under general anesthesia is advocated if an attempt without anesthesia has been unsuccessful, except in the case of an elderly primigravida or medical contraindications. The dangers of using general anesthesia are enumerated. In this country, the risk to the infant and mother does not justify the use of this procedure.

3. External version is used in the treatment of transverse lies, but Cesarean section is not resorted to if version fails.

4. Induction of premature labor as a possible course of action in the management of contracted pelvis is mentioned, but "is reserved for multigravidae with no more than first degree disproportion." The definition of first-degree disproportion is not given.

5. Laparotomy for ectopic pregnancy is begun under saline infusion, blood being given only after bleeding has been controlled.

These differences and the superficial treatment of many of the various aspects of prenatal care severely limit the value of this book in America.

ROBERT T. MILLER

A Glossary of Mycology. By W. H. Snell and E. A. Dick. Cambridge, Mass., Harvard University Press, 1957. xxi, 171 pp., 15 plates. $5.00.

This glossary of mycology contains almost 7,000 terms with which mycologists and research workers, as well as students, come in contact. Technical terms are defined. Although a person unfamiliar with basic mycological terms may have to refer to several other definitions in this book before understanding fully that of the original word looked up, the definitions seem to escape the sin of being circular. Greek and Latin derivations are given for the technical terms and are especially helpful and interesting. Illustrating various technical terms concerning characteristics of pilei, stipes, fructifications, etc., are 191 very clear and attractive line drawings by H. A. C. Jackson contained in 15 plates at the front of the book. In addition to the large number of technical terms defined, definitions are given for a great many other terms which one may come across in mycological work, e.g., popular (Indian Soap, Dyer's greenweed), obsolete (acrogonidium). Color terms are also defined with reference to *Repetoire de Couleurs,* Ridgway's *Color Standards,* Maerz and Paul's *Dictionary of Color,* and Saccardo's *Chromotaxia.* The more recent color code of Villalobos is not mentioned.

The book is initiated with the quotation from Samuel Johnson.

> "Every other author may aspire to praise;
> the lexicographer can only hope to escape reproach."

On the contrary, this book is quite praiseworthy; it is a needed mycological reference and required a great deal of time and effort on the part of the authors.

ELIZABETH W. MAC ARTHUR

THE INDEX

TO THIS VOLUME HAS BEEN REMOVED FROM THIS POSITION AND PLACED AT THE BEGINNING OF THE FILM FOR THE CONVENIENCE OF READERS.

1. The section on blood groups and RH factors is limited, and the management of erythroblastosis is discussed only in relation to hydrops fetalis.

2. In breech presentations, external cephalic version under general anesthesia is advocated if an attempt without anesthesia has been unsuccessful, except in the case of an elderly primigravida or medical contraindications. The dangers of using general anesthesia are enumerated. In this country, the risk to the infant and mother does not justify the use of this procedure.

3. External version is used in the treatment of transverse lies, but Cesarean section is not resorted to if version fails.

4. Induction of premature labor as a possible course of action in the management of contracted pelvis is mentioned, but "is reserved for multigravidae with no more than first degree disproportion." The definition of first-degree disproportion is not given.

5. Laparotomy for ectopic pregnancy is begun under saline infusion, blood being given only after bleeding has been controlled.

These differences and the superficial treatment of many of the various aspects of prenatal care severely limit the value of this book in America.

ROBERT T. MILLER

A Glossary of Mycology. By W. H. Snell and E. A. Dick. Cambridge, Mass., Harvard University Press, 1957. xxi, 171 pp., 15 plates. $5.00.

This glossary of mycology contains almost 7,000 terms with which mycologists and research workers, as well as students, come in contact. Technical terms are defined. Although a person unfamiliar with basic mycological terms may have to refer to several other definitions in this book before understanding fully that of the original word looked up, the definitions seem to escape the sin of being circular. Greek and Latin derivations are given for the technical terms and are especially helpful and interesting. Illustrating various technical terms concerning characteristics of pilei, stipes, fructifications, etc., are 191 very clear and attractive line drawings by H. A. C. Jackson contained in 15 plates at the front of the book. In addition to the large number of technical terms defined, definitions are given for a great many other terms which one may come across in mycological work, e.g., popular (Indian Soap, Dyer's greenweed), obsolete (acrogonidium). Color terms are also defined with reference to *Repetoire de Couleurs,* Ridgway's *Color Standards,* Maerz and Paul's *Dictionary of Color,* and Saccardo's *Chromotaxia.* The more recent color code of Villalobos is not mentioned.

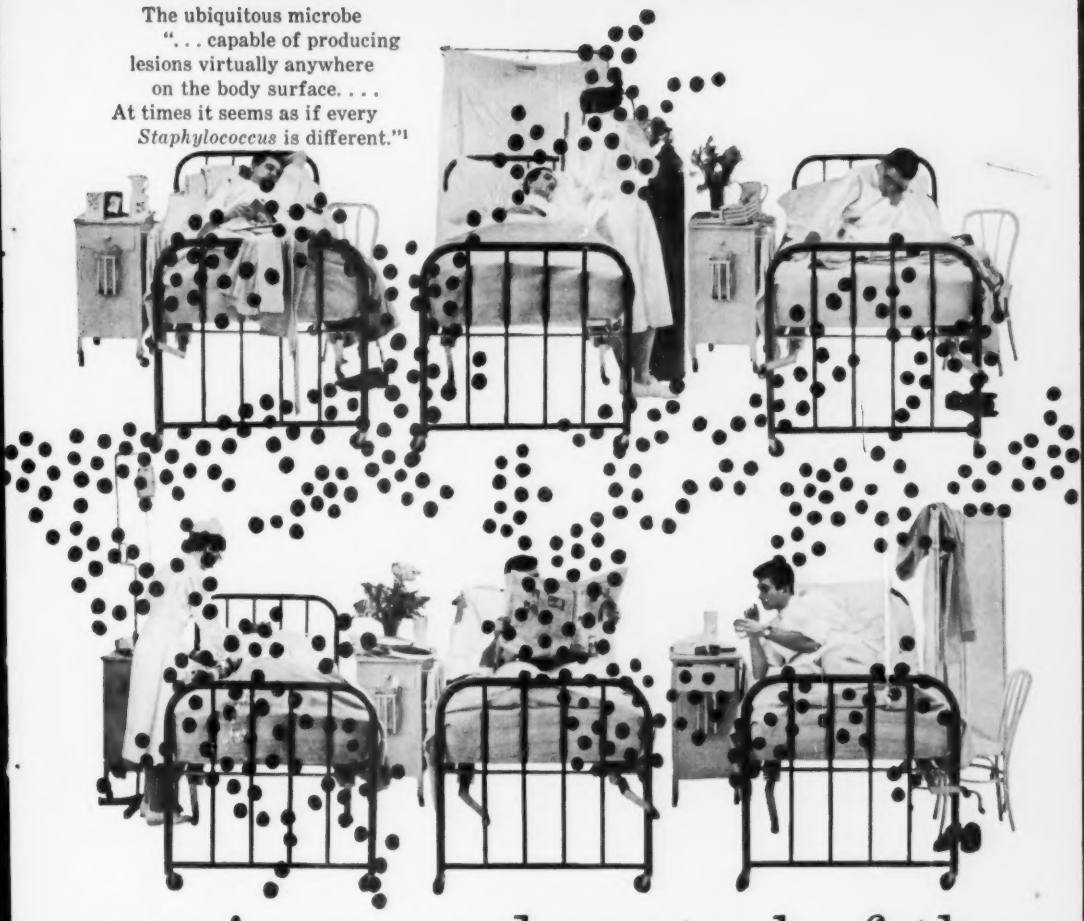
The book is initiated with the quotation from Samuel Johnson.

> "Every other author may aspire to praise;
> the lexicographer can only hope to escape reproach."

On the contrary, this book is quite praiseworthy; it is a needed mycological reference and required a great deal of time and effort on the part of the authors.

ELIZABETH W. MAC ARTHUR

The ubiquitous microbe
". . . capable of producing
lesions virtually anywhere
on the body surface. . . .
At times it seems as if every
Staphylococcus is different."[1]



now improved control of the ubiquitous Staph. with new

MATROMYCIN®

BRAND OF OLEANDOMYCIN

for the common bacterial infections that you treat with antibiotics other than broad spectrum . . . clinical success even in cases of antibiotic-resistant staphylococci

- no predictable cross resistance with penicillin, erythromycin, streptomycin, tetracycline, oxytetracycline and chlortetracycline
- resistance to Matromycin itself does not readily occur and emerges slowly and in adaptive fashion, as shown by experiments with various strains of *M. pyogenes* (clinical isolates)[2]
- outstandingly safe and well tolerated

Available in 250 mg. capsules, bottles of 16

references: 1. McDermott, W.: Ann. New York Acad. Sc. *65*:59 (Aug. 31) 1956. 2. Noyes, H. E ; Nagle, S. C., Jr.; Sanford, J. P., and Robbins, M. L.: Antibiotics & Chemother. *6*:450 (July) 1956.

Pfizer

PFIZER LABORATORIES, Brooklyn 6, N. Y.
Division, Chas. Pfizer & Co., Inc.

World leader in antibiotic development and production

Kodak reports to laboratories on:

news that only a biochemist can truly appreciate . . . modifying the spectral distribution of radiant energy . . . freezing patterns written at hundreds of miles per second

A selective precipitant for Na^+

The radio and TV newscasters have apparently failed to grasp the importance of this news (very few of them being biochemists), but word has very recently been received that *DL-Methoxyphenylacetic Acid* is a selective precipitant for sodium in the presence of large amounts of K^+, Ru^+, NH_4^+, Mg^{++}, Cl^-, NO_3^-, PO_4^{---}, SO_4^{--}, and up to 30 mg per ml of Li^+. And we gladly sell it as Eastman 7361, even providing an abstract if you like.

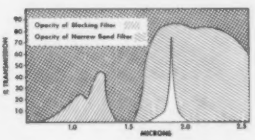
We first heard of DL-Methoxyphenylacetic Acid *as a plant growth regulator, but it wasn't spectacular. Now its new use makes it a pleasure to add it to our stock of some 3500 Eastman Organic Chemicals. For the abstract, chemical, or a copy of our List No. 40, write Distillation Products Industries, Eastman Organic Chemicals Department, Rochester 3, N. Y. (Division of Eastman Kodak Company).*

Filters: plea and offer

Once every few years a queer sense of obligation compels us to spend our hard-earned money on advertising to acquaint the next cadre of technical people with the existence of *Kodak Wratten Filters*. Of these precisely dyed little sheets of gelatin we offer at present 116 different species, and though in truth they make little direct contribution to our prosperity they do seem to contribute to the convenience of those who desire to modify the spectral distribution of radiant energy by simple and reasonably reproducible means.

The data book "Kodak Wratten Filters," which describes them all in the fullest, most quantitative spectrophotometric and colorimetric detail, has recently appeared in its 19th edition. Kodak dealers sell it for 75¢. The one niggardly favor we would ask in return for our magnanimity in selling the filters is that before an order is placed, this edition, and not one of its 18 predecessors, be consulted for current specifications and designations.

As for the newer-fangled and much costlier multi-layer interference filters, we make them but so also do several of our distinguished optical-manufacturing contemporaries. We shall not feel at all badly about your giving them your custom if you need transmission in the range up to 1.4μ. But if you find it necessary to specify a wavelength between 1.4μ and 4.5μ as a narrow transmission band or



any wavelength above 1.4μ as a cut-off for a blocking filter—there we think you need us.

In that event, just state your problem to Eastman Kodak Company, Military and Special Products Sales Division, Rochester 4, N. Y.

Oscillographic opinion

Call us ingenuous (if not ingenious). Still it's easy to be amazed that it has become humdrum routine for patterns to be written at hundreds of miles per second and then frozen nigh forever for the minutest examination and debate. (Even the seemingly agile stream of electrons in your TV picture tube plods its monotonous course at scarcely 3½ miles to the second.)

The credit is not all ours. The manufacturers of oscillographs have contributed nobly to the feat. One of them recently was kind enough to offer us the rostrum of his company publication to vent our latest opinions about film for high speed oscillography.

Our opinions are that

1. It is going to be extremely difficult to improve on the speed and image quality you get from simply developing *Kodak Tri-X Film* in *Kodak Developer D-19* at 68 F for 10 minutes. (True, the new *Kodak Royal-X Pan Film* enjoys a fourfold speed advantage over *Kodak Tri-X Film* for picture taking, but a force-developed line-image that's just over the threshold between being there and not being there is an entirely different proposition from picture taking.)
2. Development for 12 minutes in *Kodak Developer SD-19a* gives higher contrast. Under some circumstances this is as good as a gain in speed. But you pay in granularity.
3. Under some conditions, an overall postexposure of 1/500 meter-candle for one second helps the oscillographic speed a little.
4. P-16 phosphors seem in many cases to be more satisfactory photographically than P-11 phosphors.

The most convenient way to get further details is to persuade Mr. L. Arthur Hoyt, Allen B. DuMont Laboratories, Inc., 760 Bloomfield Avenue, Clifton, N. J., that your technical sophistication entitles you to a place on the mailing list for "DuMont Instrument Journal," beginning with the May, 1957, issue.

Price quoted is subject to change without notice.

This is one of a series of reports on the many products and services with which the Eastman Kodak Company and its divisions are . . . *serving laboratories everywhere*

Kodak
TRADE MARK